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The recovery and characterization of organic micropollutants from Missouri subsurface waters

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Department:

THE RECOVERY AND CHARACTERIZATION OF ORGANIC MICROPOLLUTANTS FROM MISSOURI SUBSURFACE WATERS

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ABSTRACT

The main objectives of this investigation were the recovery and partial characterization of organic micropollutants from Missouri subsurface waters and the evaluation of the number of filters in series required for effective removal of organic materials from water using the Carbon Adsorption Method.

The organics were recovered from a spring and two deep wells using three large capacity (1.5 cu. ft.) activated carbon filters in series. Chloroform and ethanol were employed as primary elutants, but acetone and benzene were also investigated. Characterization was by organoleptic, chemical, and biological determinations, toxicity measurements, and solubility partitioning.

Organic materials were found in subsurface waters ranging in concentrations from a low of 2.15 μg/l to a high of 290 μg/l total chloroform and alcohol soluble extracts in a well and spring, respectively. The extracts from the various subsurface sources exhibited considerably different characteristics from the extracts recovered in a similar manner from surface waters. All the organics exhibited an odor potential; the chloroform soluble extracts had a greater potential than the corresponding alcohol soluble materials, and the spring extracts were considerably more odorous than the well extracts. The spring extracts appeared to be biodegradable to a limited extent, while the well extracts were not. Although none of the extracts evaluated inhibited the activity of unacclimated activated sludge microorganisms, the combined chloroform and alcohol extracts from the spring showed acute toxicity to fish at a concentration of 130 μg/l.

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I. INTRODUCTION

In recent years, many investigators have become increasingly aware of the presence of organic micropollutants in surface waters. These organic materials are found not only in polluted streams receiving industrial and domestic wastes, but also in apparently unpolluted waters. They originate from several pollutional sources such as domestic and industrial wastes, agricultural runoff containing pesticide and fertilizer residuals, and accidental spillage. In addition, biologically resistant metabolic products are contributed by the microbial population of virgin and polluted streams.

Although these organic micropollutants are present in water in trace quantities which are in the microgram per liter range, they are readily detected by the tastes and odors they impart to the water. Of considerable importance may also be the long term toxic effects of these organic materials as indicated by the recovery of carcinogenic organic substances from river water receiving industrial pollution in Japan (1) and from river water used for drinking purposes and deep soil deposits in Germany (2). The large scale fish kills in the lower Mississippi River in the past several years have been also attributed to the build up of organic pesticide residuals in the fish (3). In addition, most of these trace organics are refractory;* consequently, their concentration may be expected to increase with the recycling of surface waters necessary to meet future demands. Therefore, investigations to identify these materials and determine

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*materials neither removed by ordinary water treatment facilities nor biodegradable to any large degree

their characteristics and long term toxicity are needed in order to evaluate water quality and develop methods for their removal or destruction.

Because of the minute concentration of these contaminants in surface waters, a method must be utilized to concentrate and recover them from the water before studies to identify and characterize these materials can be undertaken. Several methods of recovering organics have been investigated including liquid-liquid extraction followed by color comparison and liquid-liquid extraction followed by identification of specific materials with gas liquid chromatography. However, the most widely used method for recovering organics is the carbon adsorption technique developed by the United States Public Health Service. This method consists of passing a known volume of water (usually 5000 gallons) through an 18 inch by 3 inch diameter cylinder filled with activated carbon at a flow rate of 1/2 gpm and eluting the organics from the carbon by serial extraction with chloroform. Three general types of modifications have been applied to the basic carbon adsorption method to increase its efficiency, including extraction with other solvents to recover materials not elutable with chloroform, use of a larger volume of carbon to allow sampling a large volume of water in a relatively short period of time, and adjustment of the pH of the water to aid adsorption. Sophisticated equipment and procedures, such as the total carbon analyser, thin layer chromatography, and gas liquid chromatography, are necessary for the identification of specific organic compounds.

The presence of nonbiodegradable detergents in many underground waters, as evidenced by foaming, indicates that other organic contaminants may be also found in these waters. Considering that the majority of underground waters are

not treated, a serious danger could exist for the individuals consuming these waters. Investigations dealing with the presence and effects of organic microcontaminants in underground water therefore appear to be very necessary.

The main objective of this investigation was the recovery of organic micropollutants from underground waters and the partial characterization of these substances by organoleptic, chemical and biological determinations, toxicity measurements, and solubility partitioning. An additional objective was the evaluation of the carbon adsorption method with regard to the number of filters required for the effective recovery of organic materials.

This investigation is part of a research study whose scope is to determine the long-term toxic effects of organic micropollutants in surface and subsurface Missouri waters and the development of methods for their removal and/or destruction.

Meramec Spring in Phelps County, Missouri, and two deep wells supplying water to the Rolla area were sampled using three large capacity carbon filters arranged in series. The organics were sequentially eluted from the carbon with chloroform, ethanol and other solvents, and characterized by solubility partitioning, elemental chemical analysis, chemical oxygen demand determinations, biodegradability studies using a Warburg respirometer, bioassays, and organoleptic studies.

II. REVIEW OF LITERATURE

The purpose of this literature review is to present investigations pertinent to the recovery of organic micropollutants from water; the subsequent characterization, identification, and evaluation of their toxic effects; and their removal from drinking water supplies.

A. RECOVERY OF ORGANIC MICROPOLLUTANTS

Organic micropollutants are found in water in trace amounts; consequently, a means of concentrating and recovering these organics from water must be utilized in order to characterize and study these materials and identify their components.

The method of recovery which has been most widely used in the United States is the carbon adsorption method (CAM) which was developed by Braus, et al. (4) at the Robert A. Taft Sanitary Engineering Center. This procedure consists of passing 3000 to 5000 gallons of water through a vertically oriented filter, 18 inches high and 3 inches in diameter, containing 9 inches of 30-mesh activated carbon between two layers of 4 x 10-mesh activated carbon each 4.5 inches thick; filtration is carried out at a flow rate of 1/4 to 1/2 gpm providing a contact time of 4.4 to 2.2 minutes, respectively. The carbon is dried and then serially eluted with chloroform to desorb the organic materials. Chloroform was chosen as the solvent because it had been shown (4) that the chloroform soluble extracts (CCE*) contained the organics which exhibited definite odor potential.

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*Carbon chloroform extracts

Several extraction cycles, each requiring approximately 30 minutes, are necessary to elute significant quantities of the adsorbed materials. According to Middleton, et al. (5), after 46 hours of serial extraction with chloroform, over 99 per cent of a test organic material (phenol) was recovered from the activated carbon. However, after only 24 hours of serial extraction, 97 per cent of all material removed in 46 hours was recovered.

The coarse carbon at each end of the filter aids in preventing clogging by mud and silt from turbid waters. Because turbid river waters frequently clog the carbon filter when attempting to sample large volumes of water, a means of pretreating the raw water is a necessity. The pretreatment facility originally used by the Taft Center investigators (4) consisted of a sand filter. This was later modified by the Taft group (5) to include a sedimentation tank before the sand filter for highly turbid waters, while sedimentation followed by diatomite filtration has been used by researchers at Washington University (6, 7, 8) in St. Louis to pretreat Missouri River water before sampling.

The CAM plays an important role in the Public Health Service Water Pollution Surveillance System (formerly the National Water Quality Network) serving as a means of recovering trace organics from surface waters. Water is sampled using the CAM at 130 stations throughout the United States to determine the concentration level of organic micropollutants in surface waters and provide organic materials for exploratory work (9). Ryckman, et al. (10) in cooperation with the Missouri River Pollution Monitoring Committee, utilized the CAM to collect organic materials at eight sampling points along an 840 mile stretch of the Missouri River. These organic materials were later characterized

by solubility partitioning, taste and odor studies, biochemical oxygen demand (BOD) and chemical oxygen demand (COD) determinations, and infrared spectrographs. The CCE concentration in Missouri River water ranged from 32 to 41 μg/l.

The CAM proposed by Braus, et al. (4) has been modified by several investigators to accomplish various experimental goals. The use of more than one filter was studied by Greenburg, et al. (11) in an attempt to improve the recovery efficiency of the CAM. The second filter was used to recover any materials which might not have been adsorbed on the first filter or which might have been dislodged from the first filter as the adsorptive capacity of its carbon was reached. It was concluded that at least two columns in series were needed.

The size of the filter used in the Water Pollution Surveillance System (9) limits the amount of organic material which can be removed from the water, and, correspondingly, the volume of water which can be sampled. The low filtration rate (1/4 to 1/2 gpm) at which the filters used in these studies should be operated requires from 8 to 14 days to filter 3000 to 5000 gallons of water, respectively. Other research investigations, however, require that a large filter be used which would allow large amounts of water to be filtered in a short period of time with a subsequent recovery of large amounts of organic material. Middleton, et al. (12) developed a "mega" sampler containing 20 times as much carbon as the original filter; this allows sampling a larger volume of water, thus having the capability of recovering significant amounts of organic materials. Rosen, et al. (13), using the "mega" filter, recovered 1600 grams of CCE by filtering several thousand gallons of water in less than 10 days. The filter contained 1.24 cu. ft. of

activated carbon, 50 per cent 30-mesh and 50 per cent 4 x 10-mesh, and was operated at a rate of 7 gpm which resulted in the same detention time as the standard filter used by the Public Health Service (PHS). Since the detention time in both the "mega" filter (operated at 7 gpm) and the standard filter (operated at 1/2 gpm) is the same, the large filter can pass the same quantity of water in one day that would require 14 days with the smaller filter. Atkins and Tomlinson (14) evaluated the daily variation of CCE in Missouri River water using a large capacity filter recharged daily. A standard PHS filter recharged every 14 days was also used as a monitor. The quantity of the CCE recovered from the daily runs was almost twice as high as that obtained from the two week run with the smaller filter. According to these investigators, the consistently higher quantities of CCE obtained with the large filter was due to the larger quantity of carbon in the filter (20 times as much), especially the extra amount of fine carbon. Also, the large filter was recharged every 24 hours; therefore, its adsorptive capacity would not likely have been exceeded. The results of this investigation would seem to indicate that the organic materials recovered with the PHS filter do not represent the actual concentration level of these materials in the surface waters. Hoadley (15) has also concluded, after reviewing the CAM data from the PHS Water Pollution Surveillance System, that the adsorptive capacity of the carbon could have been exceeded and recommended that the amount of carbon should be increased or the flow rate decreased.

As it was previously mentioned, chloroform was originally chosen as the elutant for the CAM because it was found that most taste and odor producing organic materials present in natural waters are chloroform soluble. However, other

solvents have been used in recent years in an effort to recover other complex organic materials adsorbed on the carbon, many of which are not chloroform soluble. Today ethanol, which was first proposed by workers at the Robert A. Taft Sanitary Engineering Center (16), is often used following chloroform elution. According to Atkins and Tomlinson (14), the ratio of alcohol soluble extracts (CAE*) to chloroform soluble extracts in Missouri River water may vary from 3/4 to 1 up to 10 to 1 with an average value of 4 to 1.

Sproul and Ryckman (17) studied the presence of organic micropollutants in a domestic waste, a polluted stream, and an unpolluted stream (one that received agricultural runoff only) and reported the following values.

	CCE µg/l	CAE µg/l	Ratio CAE/CCE
Domestic Waste	16,000	48,000	3/1 ~~4/1~~
Polluted Stream	120	300	2.5/1
Unpolluted Stream	98	230	2.3/1

It may be noted that while in the domestic sewage the extracts were present in the milligrams per liter (mg/l) range, those in the stream waters were in the micrograms per liter (µg/l) range.

Myrick and Ryckman (6) have studied the use of two other solvents, benzene and acetone, in desorbing organic materials from activated carbon. Sequential elution with a chloroform-ethanol-acetone-benzene extraction series recovered 105 µg/l acetone soluble and 84 µg/l benzene soluble materials, in

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*Carbon alcohol extracts

addition to 81 μg/l chloroform soluble and 317 μg/l ethanol soluble extracts. When the acetone-benzene sequence was reversed, 184 μg/l benzene soluble and 35 μg/l acetone soluble extracts were recovered, in addition to the same amounts of chloroform and alcohol soluble materials.

Hamilton (18) has experimented with a mixed solvent system in order to improve the extraction of the activated carbon. It consisted of a 47 per cent propylene dichloride and 53 per cent methanol mixture and was used to study the recovery of ABS, phenol, and dichlorophenol. It was found that with the propylene dichloride-methanol mixture recoveries ranged from 84 to 95 per cent, while with chloroform they ranged from 51 to 74 per cent. According to Hamilton, in addition to giving better recoveries than chloroform, the experimental mixture was also compatible with wet carbon thereby eliminating the need for drying time and equipment.

Another modification which has been applied to the CAM is acidification of the water before filtration. Middleton, et al. (5) studied the concentrations of CCE collected under acidic conditions from tap water in Cincinnati, Ohio, and reported that the recovery under these conditions was usually equal to or greater than that under natural pH conditions. Several investigators at Washington University in St. Louis (6, 7, 8,) have also acidified the water before it was charged to the carbon filters. Myrick and Ryckman (6) recovered 81 μg/l CCE and 317 μg/l CAE from Missouri River water under its natural pH; however, when the pH was adjusted to the value of 2.0, the CCE dropped to 67 μg/l and the CAE increased to 552 μg/l.

A recent development in the removal of trace organics by carbon adsorption is the use of a fluidized carbon column (19). The primary advantage of the fluidized column is that adsorption is not hindered by highly turbid waters due to the fact that the turbidity particles are able to pass through the carbon column; consequently, they do not clog the filter. Although the main interest thus far has been the development of a tertiary waste treatment method for the removal of persistent organic pollutants, this method has the capability of being used to recover trace organics from water using a procedure similar to the CAM.

Even though the CAM is the most widely used method in the United States for recovering organic materials from water, it does have the limitations that all the organic materials present in the water may not be adsorbed on the carbon and all of those adsorbed may not be recovered with the solvents used. Middleton, et al. (5) studied the adsorption of phenol on activated carbon and its subsequent desorption with chloroform and other solvents. It was found that the adsorption efficiency ranged from 30 to 99 per cent, the desorption efficiency from 61 to 77 per cent, and the overall recovery from 19 to 77 per cent. Hoak (20), on the other hand, reported that phenol adsorption on activated carbon was high, in all cases not less than 94 per cent, while desorption was as low as 22 per cent. He suggested that chemical changes in the adsorbed phenol were the causes for this low efficiency.

Several other methods have also been proposed for the recovery of organic materials from water. Among these, liquid-liquid extraction has received considerable attention, primarily by foreign investigators. Although this method

is simpler than the CAM, a higher concentration of organics is usually necessary for this procedure to work efficiently. It is for this reason that its major use lies in recovering materials from wastes, both domestic and industrial.

Morris, et al. (21) was the first group to employ liquid-liquid extraction; they extracted organics from water with carbon tetrachloride and then identified them by color comparison with blanks of known materials and of known concentration. Other investigators (22) have also used the method of Morris, et al. to recover trace amounts of benzene, toluene, and styrene from waste waters. This method does detect and identify specific organic materials but does not furnish an organic extract which can be subjected to further investigation.

Several investigators (23, 24, 25) have used liquid-liquid extraction to recover organic materials which were then subjected to various forms of identification (gas liquid chromatography, thin layer chromatography, and elemental chemical analysis). The procedure was basically the same as that of Morris, et al. (21) except that a larger size sample was used in order to recover as much organic material as possible, a higher sample to solvent ratio was employed, and the solvent containing the organic materials was evaporated to provide an organic extract. [For example, 10 liters of sample and a sample to solvent ratio of 10 to 1 were used by Harlin, et al. (26) as compared with 100 ml of sample and 4 to 1 sample to solvent ratio used by Morris, et al. (21).]

Liquid-liquid extraction may be performed on a batch basis (26) or on a continuous flow basis as recently proposed by Sanderson and Ceresia (27) who reported that the continuous flow liquid-liquid extraction process can be used to recover organic compounds, particularly the chlorinated aromatic pesticides,

at concentrations in the μg/l range or less. They also stated that the efficiency of the process was not less than 95 per cent.

In a recent review of new techniques for the evaluation of organic micropollutants, Ryckman, et al. (28) pointed out that while liquid-liquid extraction gives greater organic material yields than the unmodified CAM and is completed in 2 to 4 hours as compared to as long as 3 weeks for the CAM, it is affected by turbidity which may form emulsions and make reproducibility difficult, requires a solvent which is immiscible in water, and limits the volume of water which can be sampled.

Baker (29) recovered various organics from water with freeze concentration in an attempt to find a better method than the CAM. He reported that recovery efficiencies varied considerably (40 per cent for 2,4-dichlorophenol to 70 per cent for m-cresol) and were not reproducible.

B. CHARACTERIZATION AND IDENTIFICATION OF ORGANIC MICROPOLLUTANTS

The characterization of organic pollutants involves several techniques. Perhaps the most widely used method is the solubility partitioning of organic extracts (9, 30). Solubility partitioning separates the organic materials into different groups on the basis of their solubility in a series of solvents. Myrick and Ryckman (6) utilized solubility partitioning to characterize CCE samples recovered from Missouri River water at St. Louis using a large capacity activated carbon filter, while Grigoropoulos and Ryckman (31) employed the method for the group breakdown of a composite CCE sample prepared from

extracts obtained over a period of one year from Missouri River water at St. Louis. The results of these investigations were as follows.

Fraction	Per Cent of Total	
	Myrick and Ryckman	Grigoropoulos and Ryckman
Ether Insolubles	9.5	2.2
Water Solubles	19.5	25.8
Amines	1.0	1.7
Amphoterics	2.8	--
Strong Acids	11.5	16.3
Weak Acids	12.8	10.0
Neutrals*	34.3	26.3
(Aliphatics)	(3.2)	(11.7)
(Aromatics)	(7.2)	(6.9)
(Oxygenated Compounds)	(87.8)	(76.5)
(Others)	(1.8)	(4.9)
Others	8.6	27.7

*Neutrals further separated by column chromatography into aliphatic, aromatic, oxygenated compounds, and others.

Middleton, et al. (5) employed solubility partitioning to characterize CCE recovered from the Cincinnati River. He reported the following breakdown.

Fraction	Per Cent of Total
Acidic	1.8
Basic	1.3
Neutral	57.5
Phenolic	24.8
Others	14.6

Myrick and Ryckman (6) also attempted to characterize CAE from Missouri River water by solubility partitioning but found that the extracts were 99 per cent ether insoluble; consequently, further separation was not attempted.

Another technique which is used to characterize organic micropollutants is the elemental chemical analysis of the extracts. Based on elemental composition of the previously mentioned organic extracts, Myrick and Ryckman (6) reported

empirical formulas of $C_{5.5}H_{7.1}O_{1.2}N_{0.06}S_{0.05}$ and $C_{2.5}H_{6.1}O_{2.1}N_{0.17}S_{0.03}$ for CCE and CAE, respectively, while Grigoropoulos and Ryckman (31) suggested a simplified empirical formula of $(C_7H_{11}O_2)_x$ for the CCE composite sample.

Goncharova and Datska (25) recently reported the results of a microchemical analysis performed on an organic extract which was recovered from well water by liquid-liquid extraction; this analysis corresponds to an empirical formula of $C_{4.4}H_{7.0}O_{2.3}N_{0.23}$.

The biochemical oxygen demand and the chemical oxygen demand have been used also as parameters for the characterization of organic extracts. Ryckman, et al. (10) reported that the 20-day BOD of CCE extracts recovered from Missouri River water ranged from 0.13 to 0.31 mg of oxygen per mg of extract and the COD varied from 2.11 to 2.35 mg of oxygen per mg of extract. Myrick and Ryckman (6) also reported that the COD values varied from 2.1 to 2.3 and from 1.2 to 1.4 mg of oxygen per mg of CCE and CAE, respectively.

The organic micropollutants extracted from aqueous solutions consist of such numerous and varied chemical compounds as to make their separation and identification an extremely difficult process, requiring the most refined and sophisticated methods. The two analytical techniques utilized with greatest success to date are chromatography and spectroscopy. Even with these techniques which have great potential for identification, prior separatory procedures are necessary.

Thin layer chromatography has been used as a separating technique prior to gas liquid chromatography (24) and as an identifying method (32); γ-BHC, dieldrin, and pp'-DDT were identified by Wheatley and Hardman (32) in England in extracts obtained by liquid-liquid extraction of rain water.

The application of gas liquid chromatography in the separation and identification of organic materials recovered from industrial and domestic waste was investigated by Sproul, et al. (33). Toluene and ortho-dichlorobenzene were identified in a chemical plant waste, while various fatty acids, from caprylic to linolinic, were found in brewery, corn refining, meat packing, paint and soap industrial waste, and domestic waste. LaMar and Goerlite (23) have also employed gas liquid chromatography to study organic acids recovered from unpolluted surface streams in California and Washington by liquid-liquid extraction and were able to identify 12 carboxylic acids.

Fluorescence spectral analysis was used by two German investigators, Borneff and Fischer (34), to identify anthracene, 1,12-benzoperylene, 3,4-benzoperylene, and other organic materials in extracts recovered from river water. It should be noted that some of these chemicals are known to be carcinogenic.

Infrared spectroscopy was used by Tengonciang (35) in his study of the biodegradation of chloroform extracts removed from the Missouri River. Spectrograms were prepared of the original organics used in the study and of the extracts obtained from the solutions used in long-term BOD studies following incubation. The spectrograms were used to identify specific groups and to judge the efficiency of the biodegradation; no significant differences in the extracts before and after incubation were noticed. Buescher, et al. (36) utilized infrared spectroscopy to measure the effects of chemical oxidation on pesticides. They were able to identify the chlorinated hydrocarbons, lindane, aldrin, and dieldrin, in concentrations of less than one nanogram.

Another use to which infrared spectroscopy readily lends itself is in conjunction with gas liquid chromatography (37). Effluents from the chromatograph are collected and then transferred to the infrared analyzer for further identification.

C. TOXIC EFFECTS OF ORGANIC MICROPOLLUTANTS

Some of the effects of organic micropollutants, such as tastes and odors in drinking water, fouling of ion exchange materials, and foaming, are established; however, relatively little is known of the long-term physiological toxicity of these pollutants. Borneff and Fischer (38) have recovered and identified specific carcinogenic agents, including 3,4-benzopyrene, 3,4-benzofluoranthrene, and 1,2-benzoperylene, from the activated carbon used in a filter employed by a municipal water plant. They concluded that if the filter had not been used, the population consuming the water would have been subjected to mg amounts of carcinogenics in one year.

In a previous study, Borneff and Kneer (39) concluded that 3,4-benzopyrene showed carcinogenic properties only in the presence of surface active agents. They further concluded that drinking water obtained from rivers subjected to contamination with waste waters containing detergents must be considered, for the present, injurious to health. In other studies on the toxicity of benzopyrene, Borneff and Kneer (40) reported that 3,4-benzopyrene is destroyed by shortwave ultraviolet irradiation. It was estimated that the daily reduction of benzopyrene in air amounted to 20 to 50 per cent. It was also reported that 3,4-benzopyrene was most sensitive when dissolved in detergents.

Hueper and Payne (41) have investigated the long-term physiological impact of certain absorbates on mice. They concluded that the absorbates were the probable cause of leukemia in some mice and of cancer in others. Sproul and Ryckman (42) investigated the toxic effects of organic extracts (CCE) on rainbow trout. The extracts were obtained from polluted and unpolluted streams, domestic sewage, and nine industrial sources. The extracts from the unpolluted stream and domestic sewage were the least toxic (no effect on fish at concentrations up to 100 mg/l), while the extracts from a soap industrial waste were the most toxic (several toxic effects at a concentration of 5 mg/l).

D. REMOVAL OF ORGANIC MICROPOLLUTANTS

Organic micropollutants have created serious taste and odor problems in surface waters resulting in large expenditures required in producing a water of acceptable quality. The fact that these organic materials remain in the streams and pass through the present day water treatment facilities practically unaltered indicates that they are resistant to biological and physical treatment. Ryckman, et al. (10) have shown at several water treatment plants along the Missouri River that the average concentration of CCE was either the same or higher in tap water than in raw river water.

Dornbush and Ryckman (7) evaluated the physiochemical removal of soluble trace organic pollutants by selected materials. The organics chosen for evaluation were the CCE and CAE recovered from Missouri River water with a large capacity activated carbon filter. Solutions were prepared which

reconstituted the proportions of the recovered organic materials in a modified Missouri River water with natural dissolved impurities. The efficiency of activated carbon, bentonite, ferric sulfate, and alum in removing these organics was evaluated in laboratory studies. Natural silt was also studied to delineate clearly the role of natural self purification. It was concluded that application of activated carbon was the only physiochemical method studied which effectively reduced odor potential and also removed substantial quantities of soluble trace organics from solution. Coagulation with alum and/or ferric sulfate was not effective in reducing the odor potential but did remove significant quantities of organic materials.

Grigoropoulos and Ryckman (31) studied the effect of chlorine and chlorine dioxide on several taste and odor producing substances in water. They found that the CCE were resistant to chemical oxidation with chlorine or chlorine dioxide at the pH value of 10.0 and the temperature range of 5 to 30^{o}C, and that treatment with either oxidant did not substantially reduce their odor potential.

Spicher and Skrinde (8) have investigated the potassium permanganate oxidation of organic extracts (CCE and CAE) recovered from Missouri River water at both the natural pH of the water and a reduced pH with a large capacity activated carbon filter. The effect of potassium permanganate on the four individual extracts and a composite sample (containing all four extracts) was studied under various conditions of pH, temperature, concentration of organic compounds, and permanganate concentration. On the basis of potassium permanganate utilization, it was found that the permanganate was more effective as an oxidizing agent under alkaline rather than under neutral conditions, and that the CCE recovered under natural pH conditions were observed to be more readily

oxidized than the other fractions. Although potassium permanganate oxidation reduced the odor potential of all extracts by an average of 50 per cent, no direct relationship was established between odor reduction and the permanganate oxidation of the organics. The authors (8) suggested that incomplete oxidation of the organic refractories yielding intermediate organic compounds of greater or lesser odor potential than the original reactants was responsible for the lack of correlation between odor potential reduction and oxidation of organic extracts.

Tengonciang (35) studied the biodegradability of CCE recovered from Missouri River water at eight points along 840 miles of the river using long-term BOD determinations. He found that the CCE were resistant to biological degradation with the degree of resistance increasing progressively downstream. He verified his findings with infrared spectrographic characterization of the extracts before and after incubation.

Myrick and Ryckman (6) investigated the biochemical degradation of trace organics (CCE and CAE) both in the presence of low concentrations of microorganisms and organics (simulating stream conditions) and in the presence of high concentrations of microorganisms and organics (an acclimated activated sludge). On the basis of long-term BOD determinations, only approximately 3 per cent of either the CCE or a composite sample containing both CCE and CAE, and none of the CAE were degraded in ten days. The slow rate of reaction indicated that these organic pollutants were very resistant to biological degradation. It was noticed that the odor potential of the CAE increased slightly in the ten days of incubation. Under the action of 2000 mg/l biological solids, the ethanol and

chloroform fractions were resistant to biodegradation although they did not inhibit the activity of the microorganisms.

As may be seen from these investigations (6, 7, 8, 31, 35), organic micropollutants in surface waters are resistant to biological degradation either in a waste treatment facility or a stream and are not removed from water by either physiochemical processes (except for adsorption on activated carbon) or chemical oxidation as used in water treatment plants.

III. MODE OF STUDY

Organic micropollutants were concentrated and recovered from subsurface waters at their natural pH using the modified carbon adsorption method. Water from a spring and two deep wells was filtered through a fixed bed of activated carbon. The carbon was then removed from the filters, dried, and sequentially extracted with chloroform, alcohol, and other solvents to recover the adsorbed organic materials. The recovered organic pollutants were characterized by solubility partitioning, elemental chemical analysis, chemical oxygen demand determination, biodegradability studies, preliminary toxicity studies, and organoleptic determinations.

A. SAMPLING LOCATIONS

Meramec Spring which is reported to be the seventh largest in Missouri (43) was chosen as the source of spring water. Flow data and characteristics of this spring are shown in Table I. According to the Missouri Geological Survey and Water Resources (43), the source of the spring is thought to include portions of an area which lies to the south, west, and southwest of the spring; the underground supply routes are connected with surface feeder routes as is evidenced by its response to both heavy rainfall and protracted periods of drought. Its flow is known to become turbid, or even muddy, soon after heavy rainfall in the surrounding area. This characteristic of the spring was observed during the final thirty days of the thirty-seven day sampling period of this investigation. This spring was chosen as a sampling location because it is subjected to surface contamination and its proximity to Rolla permitted close observation

Table I

Sampling Location Data

Sampling Point	Location	Rock Formation	Spring Data† Ave. Flow cfs	Spring Data† Max. Flow cfs	Spring Data† Min. Flow cfs	Well Data†† Depth ft.	Well Data†† Capacity
Meramec Spring	9 miles east of St. James, Missouri on Route 8	Van Buren Dolomite	120	400	89		
UMR Well #2	At east end of UMR drill field	Potosi Dolomite				1,150	336 gpm w/26 ft. drawdown
Rolla Well #3	900 E. Arkansas Rolla, Missouri	Lamotte Sandstone				1,745	400 gpm w/33 ft. drawdown

† Data obtained from United States Geological Survey, Water Resources Division (44).

†† Data obtained from Missouri Geological Survey and Water Resources (43).

of the equipment and evaluation of procedures. In addition, another investigation was in progress at the University of Missouri at Rolla (UMR) (45, 46) to evaluate the quality of Meramec Spring water.

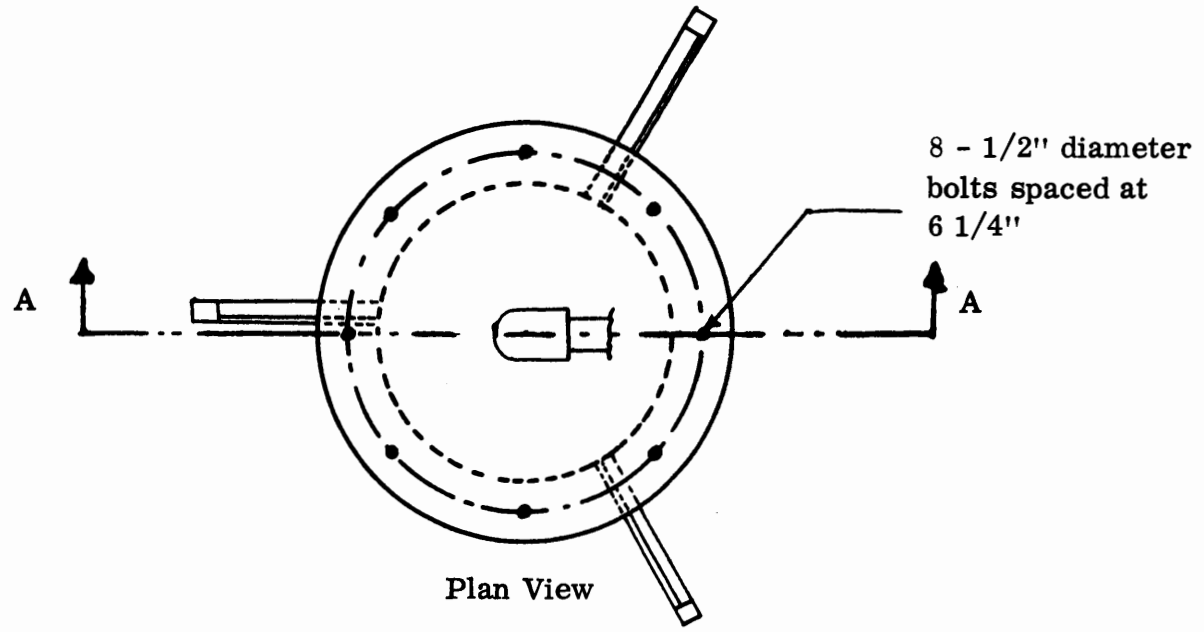
The second sampling source was a deep well located on the UMR campus which had been used as a source of drinking water. The well is presently capped and no longer used because of high coliform counts and large amounts of turbidity which appeared immediately after the Alaskan earthquake (March 1964) and which suggested possible contamination by surface water. This well was chosen as part of the sampling program because it was not in use as a drinking water source and could be pumped continuously allowing a short, continuous sampling period. Pertinent data for this well are shown in Table I.

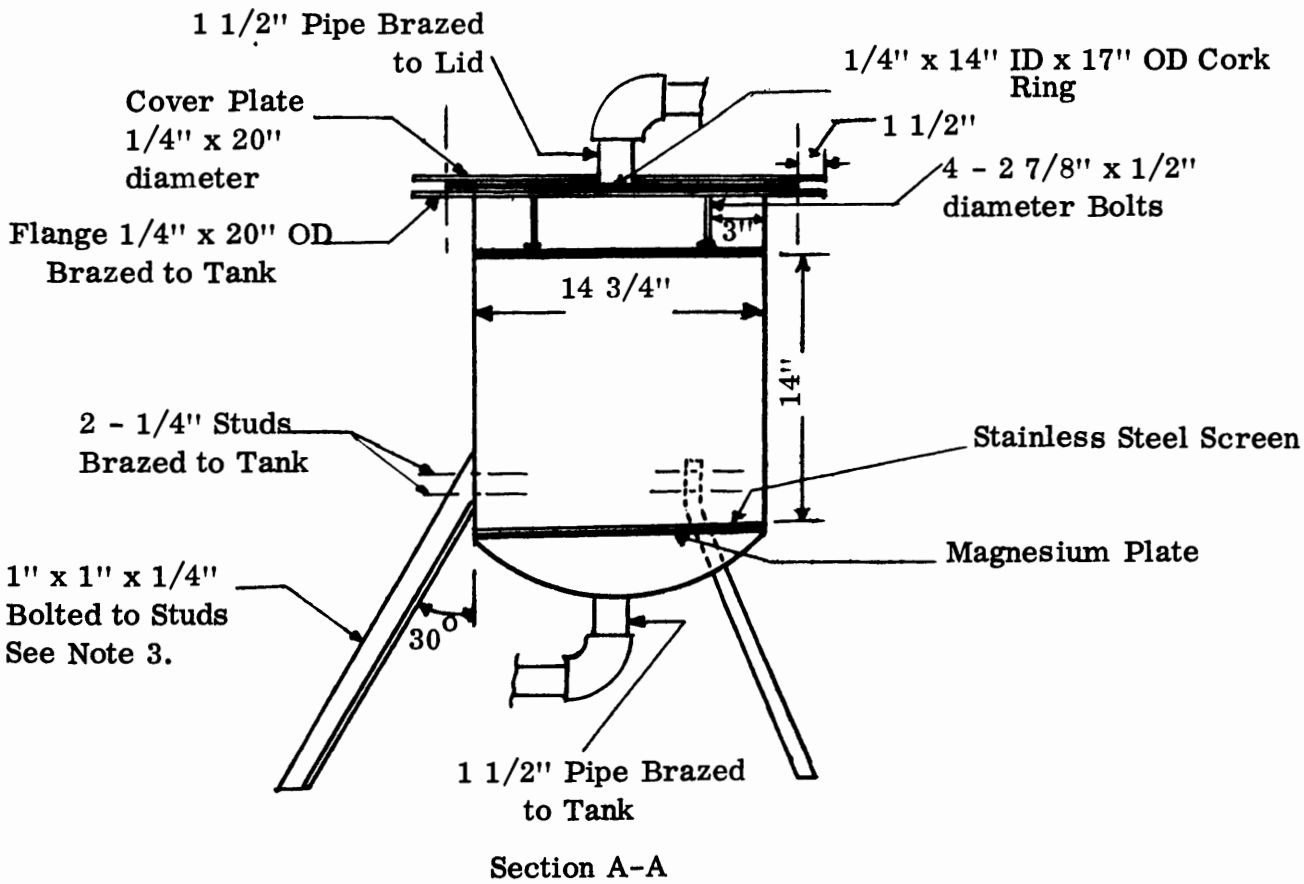
In an attempt to obtain more data on deep subsurface water, a deep well used by the City of Rolla to supply part of the city's water demand was selected as the third sampling location. This well has no known source of surface contamination, but according to city officials (47) periods of high turbidity have occurred in the past. The City of Rolla has nine wells in use and alternates the pumping of each in order not to exert an excessive demand on any one well. The well was sampled by tapping into the discharge line of the pump and using a series of valves to control the flow rate and pressure. Due to the city's alternate pumping program, the activated carbon filters were operated intermittantly which greatly increased the sampling period. Well data for this well are shown in Table I.

B. CONCENTRATION OF ORGANICS WITH CARBON ADSORPTION

In order to recover significant amounts of the organic materials present in the sources studied, a large volume of water was passed serially through three large capacity activated carbon filters each containing 1.525 cu. ft. of carbon. Three carbon filters were used in an attempt to recover all the organic micropollutants found in the water. The second and third filters were used to recover any materials not adsorbed on the first filter and those materials leached from the first filter due to its adsorptive capacity being exceeded. The carbon filters were constructed from one-half of a 100 pound propane gas bottle to which were attached a lid and influent and effluent pipes. The dimensions and construction details of a typical filter are shown in Figure 1. The propane gas half bottle was selected as the basic part of the filter because it was made of steel (providing an adequate pressure vessel) and was of sufficient size to hold a large volume of activated carbon. The inside of the steel tank was sand blasted to remove the residual paint and rust and then coated with two coats of epoxy primer (Phelan-Faust #4051) followed by three coats of white epoxy enamel (Phelan-Faust #3850). Epoxy paint was selected as a coating for the sand blasted steel to provide an inert surface resistant to corrosion due to wet carbon. Two 40 x 40 mesh stainless steel screens, supported by perforated magnesium plates, were placed on the top and the bottom of the carbon column to keep the carbon in a packed state.

The filter arrangement employed at each sampling location (Figure 2) was the same except for the type of pump used to supply water to the filters. For the





Section A-A

Notes:

1. All brazed joints to be pressure tight.
2. Scale 0.01" = 1.0"
3. Three legs spaced at 15 1/2".

Figure 1

Activated Carbon Filter

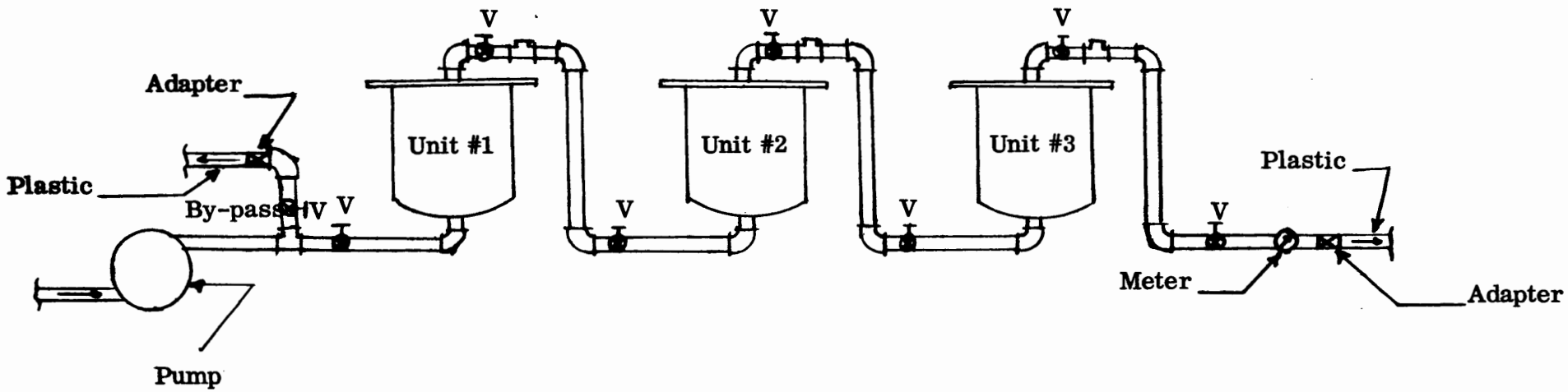


Figure 2

Activated Carbon Filter Arrangement

Scale 0.01" = 1.0"

Note: All pipes 1 1/2" galvanized iron except as noted.

spring samples, water was supplied continuously to the filters with a 1.5 horsepower centrifugal pump. The flow rate and pressure were regulated with a series of valves and with by-passing the excess flow. The inlet facility consisted of a 1.5 inch diameter galvanized pipe extending into the mouth of the spring. The filter set up and arrangement used at Meramec Spring is shown in Figure 3. For both well sampling locations, water was supplied to the filters with the deep well impeller-type pump used to draw water from the well during normal operation. At each well, water was obtained from the effluent pipe of the pump before the water was chlorinated. As was previously mentioned, the filters at the UMR well were operated continuously with the excess water being wasted to a storm sewer, while at the City of Rolla well the filters were operated only when the pump was running with the excess water going into the city's water storage facilities. At both wells, a series of valves was used to reduce the pressure to the desired operating level and flow rate. The filter arrangement employed at the UMR well is shown in Figure 4. The total volume of water filtered during each filter run was measured with a standard impeller-type water meter furnished by the City of Rolla Water Department.

The selection of the flow rate and the total volume of water to be filtered was based on several factors. The volume of water which could be filtered was limited by the adsorptive capacity of the activated carbon and the desired length of the filter run. Also considered was that low flow rates were desirable for efficient adsorption, but the lower the flow rate the longer would be the time required to recover the organic materials. The works of several investigators

Figure 3

Activated Carbon Filter Arrangement at Meramec Spring

Figure 4

Activated Carbon Filter Arrangement at UMR Well

were reviewed in an attempt to obtain a general range of flow rates and sample volumes. A summary of these works is shown in Table II. On the basis of the results of these studies and in consideration of the nature of the sources to be sampled, the total volume of water to be filtered and the operating flow rate were chosen and are presented in Table III.

In selecting the volume and types of activated carbon to be used, the works of several investigators shown in Table II were considered. Cliffchar 4 x 10 mesh and Nuchar C-190 + 30 mesh were chosen as the coarse and the fine activated carbon, respectively. Several characteristics of these carbons were determined and are presented in Table IV. The total volume of carbon per filter was 1.525 cu. ft. and consisted of 50 per cent coarse and 50 per cent fine. The coarse carbon was divided into two equal layers, one on the top and one on the bottom of the fine layer.

The filters were packed at the Civil Engineering Building and then transported to and assembled at the sampling location. All the pipes, valves, and connections employed were cleaned with kerosene and hot water to remove any organic contaminants. Epoxy paint was used as a pipe thread sealant to provide an inactive, but not permanent seal. Due to the large amount of fines present in the activated carbon, it was necessary to wash sequentially each filter before starting a filter run until all the fines were removed. The procedure followed during a filter run was to adjust the flow rate to the desired amount using the valve preceeding filter number 1 and that following filter number 3 and observe the flow rate and pressure continuously for the first three hours. Thereafter,

Table II

Slected Data from Previous Investigations
Using the Carbon Adsorption Method

Investigators	Volume of Activated Carbon cu. ft.	Carbon Size†	Flow Rate gpm	Detention Time min.	Total Volume Filtered gal.	Volume per cu. ft.
Washington University Group (6, 7, 8)	1.30	4x10 mesh (40%)†† +30 mesh (60%)	5.0-7.5	4.0-6.0	96,000	73,800
Taft Center Group (12, 13)	1.24	4x10 mesh (50%)†† +30 mesh (50%)	3.5	2.8	99,400	80,000
Braus, et al. (4)	0.073	4 x 10 mesh (40%)†† +30 mesh (60%)	0.25-0.50	2.2-1.1	5,000	68,500

†Coarse carbon - Nuchar C-190
Fine carbon - Cliffchar
††Two equal layers, one on top and one on bottom of fine layer.

Table III

Data for Activated Carbon Filter Runs

Sampling Point	Sampling Period	Number of Sampling Days	Flow Rate Ave. gpm	Flow Rate Max. gpm	Flow Rate Min. gpm	Volume Filtered gal.
Meramec Spring						
Run #1	Dec. 23, 1965 to Jan. 9, 1966	17	5.46	6.80	4.80	129,036
Run #2	Jan. 9, 1966 to Feb. 1, 1966	18	4.87	5.50	4.80	132,777
UMR Well #2	Jan. 17, 1966 to Feb. 1, 1966	15	6.84	10.00	6.30	142,360
City of Rolla Well #3	March 26, 1966 to May 31, 1966	115†	6.23	8.40	8.31	262,301

† Pump was operated 38 days (equivalent to 35 24-hour days) during this period.

Table IV

Characteristics of Activated Carbon

CLIFFCHAR 4 x 10 mesh	
Sieve Analysis	
% Retained on #4	10%
% Retained on #10 but passing #4	80%
% Retained on #20 but passing #10	10%
Chloroform Extractable Materials	0.013 mg/gram carbon
Alcohol Extractable Materials	0.360 mg/gram carbon
Acetone 1 Extractable Materials	0.010 mg/gram carbon
Acetone 2 Extractable Materials	0.008 mg/gram carbon
Benzene 1 Extractable Materials	0.081 mg/gram carbon
Benzene 2 Extractable Materials	0.094 mg/gram carbon
NUCHAR C-190 +30 mesh	
Sieve Analysis	
% Retained on #20	5%
% Retained on #30 but passing #20	20%
% Retained on #40 but passing #30	40%
% Retained on #60 but passing #40	35%
Chloroform Extractable Materials	0.052 mg/gram carbon
Alcohol Extractable Materials	0.900 mg/gram carbon
Acetone 1 Extractable Materials	0.050 mg/gram carbon
Acetone 2 Extractable Materials	0.040 mg/gram carbon
Benzene 1 Extractable Materials	0.241 mg/gram carbon
Benzene 2 Extractable Materials	0.300 mg/gram carbon

Note: The numbers "1" and "2" refer to the order in which acetone and benzene were used to elute the carbon following chloroform and alcohol extraction.

the flow rate was checked both by direct observation and by calculation using the volume of water filtered over a period of time each day for the first eight days and then every other day for the remainder of the filter run with the flow rate adjusted when necessary. The total quantity of water filtered was recorded each time the flow rate was checked. After the desired volume of water had been filtered (Table II), the pump was stopped, the filter arrangement disconnected, and the filters allowed to drain; the carbon was then removed by hand and placed in 14 x 28 inch plastic bags for transportation to the laboratory. At the laboratory the activated carbon was placed in 24 x 36 x 3 inch wood trays lined with 4 mil polyethylene sheets; the trays were placed in a walk-in incubator; and the carbon was allowed to dry at 40°C for 3 to 5 days with carbon-filtered air circulating through the incubator. The carbon was stirred twice daily during the drying period. After the carbon was dry, it was removed from the incubator and placed in the plastic bags for storage. Blanks were prepared by wetting virgin carbon with deionized water, placing the wet carbon in plastic bags for two hours, drying the carbon on polyethylene sheets, and then storing it in the plastic bags.

C. RECOVERY OF THE ORGANIC MATERIALS

After the carbon had been dried, it was subjected to serial extraction with various solvents to remove the adsorbed organic materials. The extractions were carried out with four modified Soxhlet extractors (Pyrex #3885) using re-distilled chloroform and ethanol (95%) as the primary solvents. Redistilled benzene and acetone were also used to investigate the amount of organic materials remaining on carbon after the CCE and CAE had been removed. The arrangement

of the extractors is shown in Figure 5. The general procedure as outlined in Standard Methods (48, p. 215) was followed for the extraction of organics. The carbon was extracted with chloroform for 24 hours (48 cycles), removed from the extractor, and placed in a fume hood until no traces of chloroform remained (about 48 hours). The chloroform-extracted carbon was then subjected to ethanol extraction for 24 hours, dried, and stored for future use. When chloroform, ethanol, benzene, and acetone were used to extract organics from the carbon, the same procedure of eluting, drying, and then eluting with the next solvent in the series was followed. Since Middleton, et al. (5) have shown that 94 per cent of all the chloroform-extractable material which would have been recovered in 46 hours was recovered in 24 hours and that extraction for an additional 12 hours gave only 2 per cent more (a total of 96 per cent) of the 46 hour extractable material; a 24 hour (48 cycles) extraction time was chosen which was 12 hours less that recommended by the PHS (9). A reduction in extraction time was considered necessary because of the considerable quantity of carbon which had to be extracted.

When the carbon had been extracted for 24 hours, the eluted materials were contained in 1.5 to 2 liters of solvent. The organics were concentrated by distilling off all but approximately 250 ml of solvent; the residual solvent was filtered through solvent-rinsed filter paper (Whatman #43) and then vacuum filtered through a fine sintered glass funnel; and finally it was evaporated on a water bath until a volume of approximately 75 ml remained. Filtration through solvent-rinsed filter paper was necessary to remove particles of carbon which had been leached from the carbon column during siphoning. The sintered glass

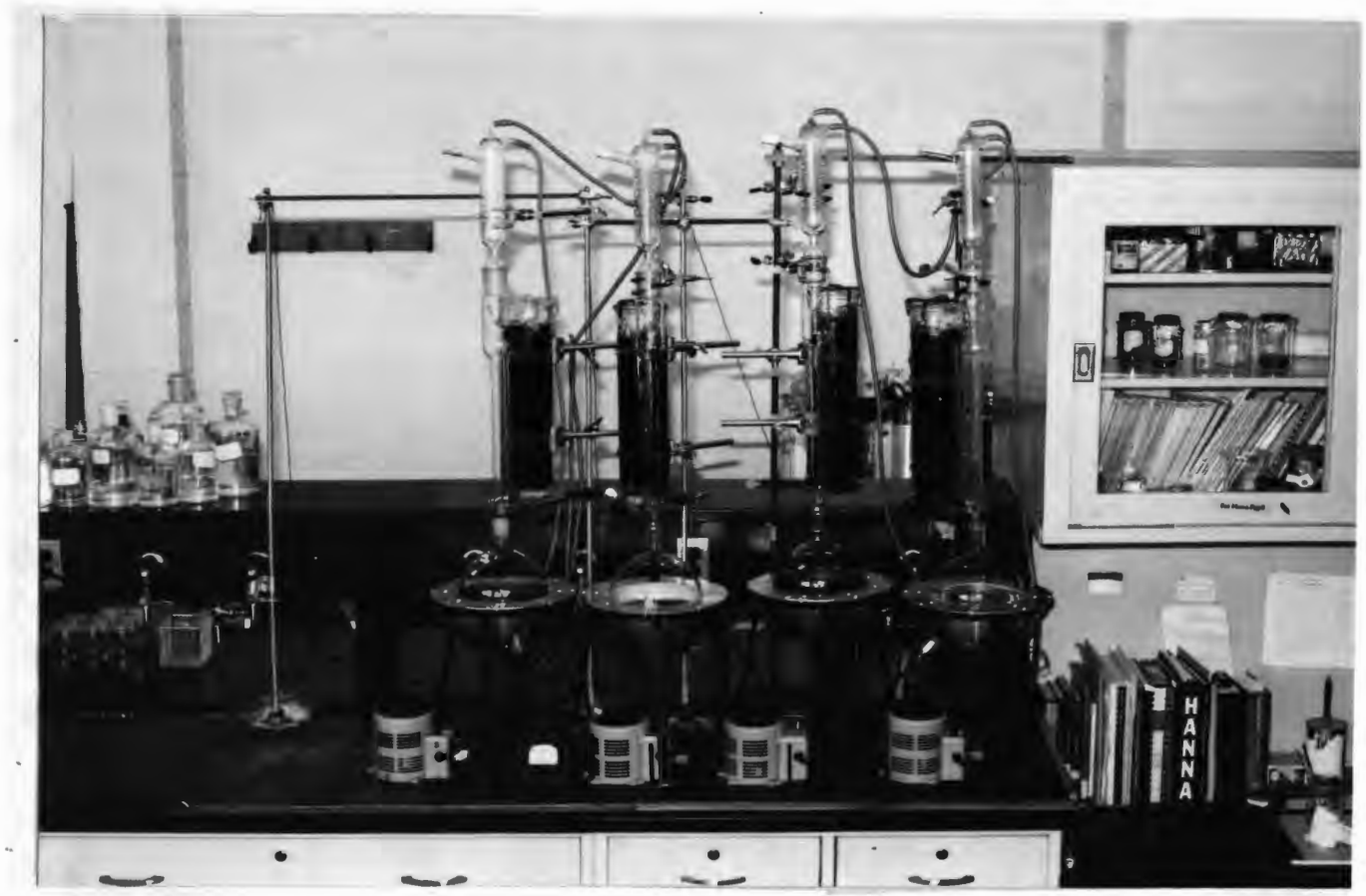


Figure 5

Modified Soxhlet Extraction Apparatus

filtration removed the carbon fines which passed through the filter paper. The remaining 75 ml of solvent containing the eluted organics was transferred to a tared 200 ml bottle before final evaporation. Because of the large volume of coarse and fine activated carbon in each filter, 20 to 22 extractions were necessary to extract one filter volume of activated carbon. The final residual 75 ml volume from each extraction was added to the tared bottle which was periodically evaporated to keep the volume of solvent in the bottle at approximately 80 ml. After all the carbon from one filter (coarse or fine) had been extracted, the remaining solvent was evaporated on a water bath with final drying in a dessicator. The solvent containing the CAE was again filtered through sintered glass before final evaporation and drying because of a large amount of white, granular material which appeared in all samples during the extraction process but could not be filtered out in the initial filtration. This white material was insoluble in organic solvents (chloroform, carbon tetrachloride, 99% ethanol, and methanol) and acids (sulfuric, phosphoric, nitric, and hydrochloric) but was very soluble in water; it appeared to be inorganic, as evidenced by the absence of any chemical oxygen demand [dilute dichromate reflux method (48, p. 512)]. The extract was then weighed to a constant weight (less than 5 mg variation for seven consecutive weighings for samples of over 10 grams). After the weight of the extracts of the same solvent for the coarse and fine carbon of a filter was determined, they were combined and stored in a 100 ml bottle.

The blanks which had been previously prepared were extracted as described above, and the amount of organic materials on the carbon was determined. These values are shown in Table IV.

D. STOCK SOLUTIONS OF ORGANICS

In order to perform the majority of the studies on the organic micropollutants, it was necessary for these materials to be in solution. Stock solutions of the CCE and CAE were made using a VirTis "Aeroseal Chemixer" (Model 24-100). A known amount of organic extract was placed in the deep fluted mixing flask with a known volume of deionized water prepared with a Barnsted Deionizer (Model BD-1 with high purity ion exchange resin) and then mixed at about 8,000 rpm until all the extract was in solution.

Due to the limited solubility of the extracts and the need for solutions of higher concentration, a large capacity Rinco Roto Evaporator (Model VE 1000) was employed to concentrate the stock solutions. Evaporation was accomplished by applying vacuum with a water aspirator and heating the solution to 45 to 50^{o}C in a water bath.

Gravimetric corrections were made, when necessary, for any material remaining in either the VirTis mixing flask or the Rinco evaporating flask.

E. PROCEDURE FOR CHARACTERIZATION STUDIES

Characterization of the recovered organics was by solubility partitioning, elemental chemical analysis, COD determinations, biodegradability studies, preliminary toxicity studies, and organoleptic determinations.

1. Solubility Partitioning.

Solubility partitioning separated the organic extract into various fractions on the basis of their solubility in ether under different pH conditions. The various fractions obtained from solubility partitioning were the ether insolubles, water

solubles, strong acids, weak acids, amphoterics, basic materials, and neutrals. The neutrals were further separated by column chromatography into aliphatic, aromatic, and oxygenated fractions. The solubility separation procedure employed is shown diagrammatically in Figure 6, while the procedure for the column chromatographic breakdown of the neutrals is given in Figure 7.

2. Elemental Chemical Analysis.

Elemental chemical analyses on several extracts were performed by the Micro Tech Laboratories, Skokie, Illinois.

3. Chemical Oxygen Demand Determinations.

The chemical oxygen demand was determined for each extract using the dilute dichromate method as outlined in Standard Methods (48, p. 512). A mixture containing 5 ml sample, 5 ml of 0.025 N potassium dichromate, 10 ml concentrated sulfuric acid containing 9.3 g/l silver sulfate, and a few pumice stones was refluxed for two hours. It was then allowed to cool, diluted to 100 ml with distilled water and the remaining potassium dichromate was titrated with 0.01 N ferrous ammonium sulfate using ferroin indicator. A reagent blank containing distilled water instead of sample was also run.

4. Biodegradability Studies.

The biodegradability studies were performed using a heated, refrigerated Gilson Medical Electronics Warburg respirometer (Model RWBP 3). The studies were conducted at 20°C, 80 to 82 oscillations, and 3.8 cm excursion of the flask. Seed microorganisms were obtained from an activated sludge unit acclimated to domestic sewage fortified with 100 mg/l glucose. Various concentrations of organic extracts (both CCE and CAE), varying from a low of 9 mg/l CCE up to

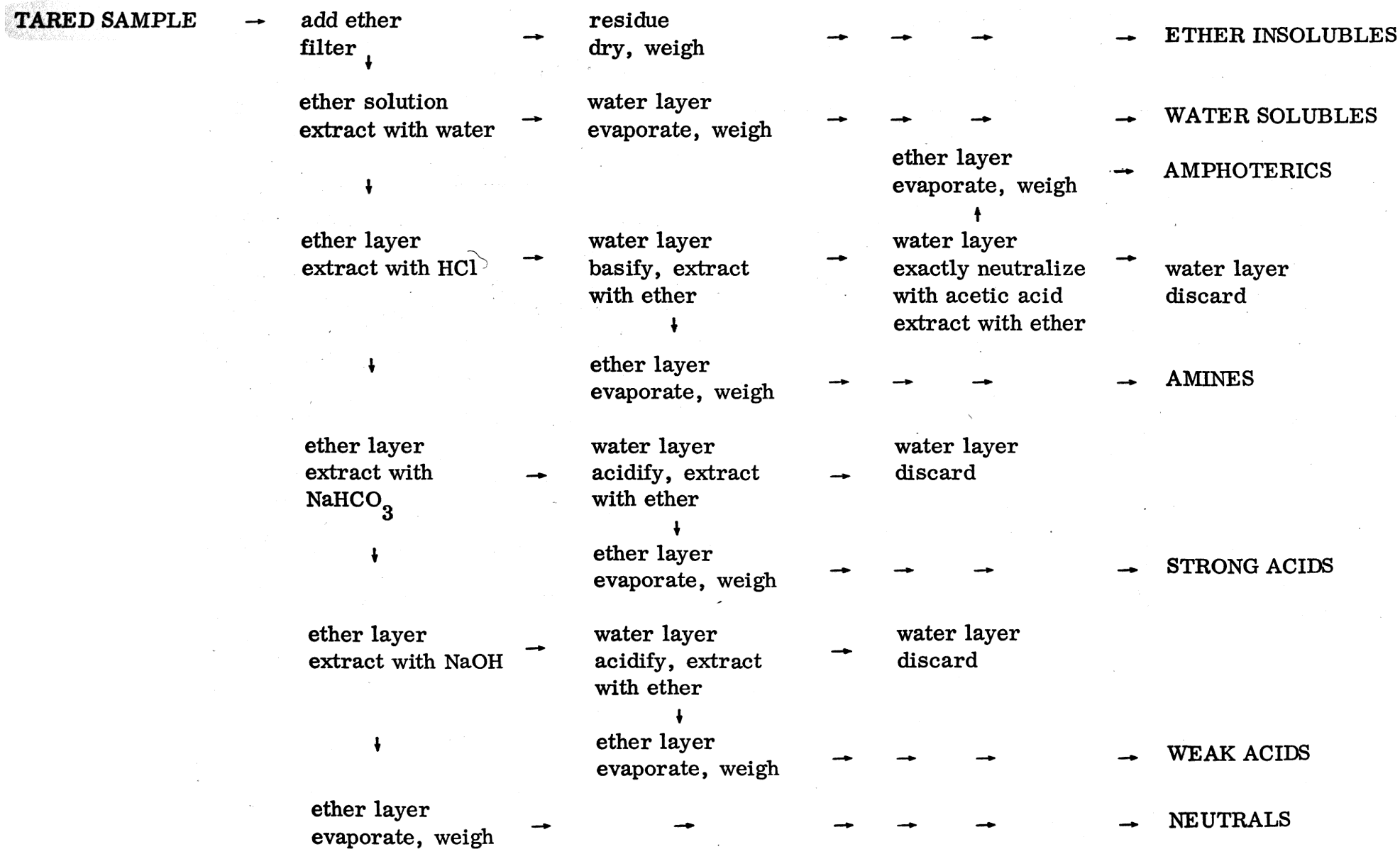


Figure 6

Scheme for Solubility Partitioning of Carbon Chloroform Extracts (30)

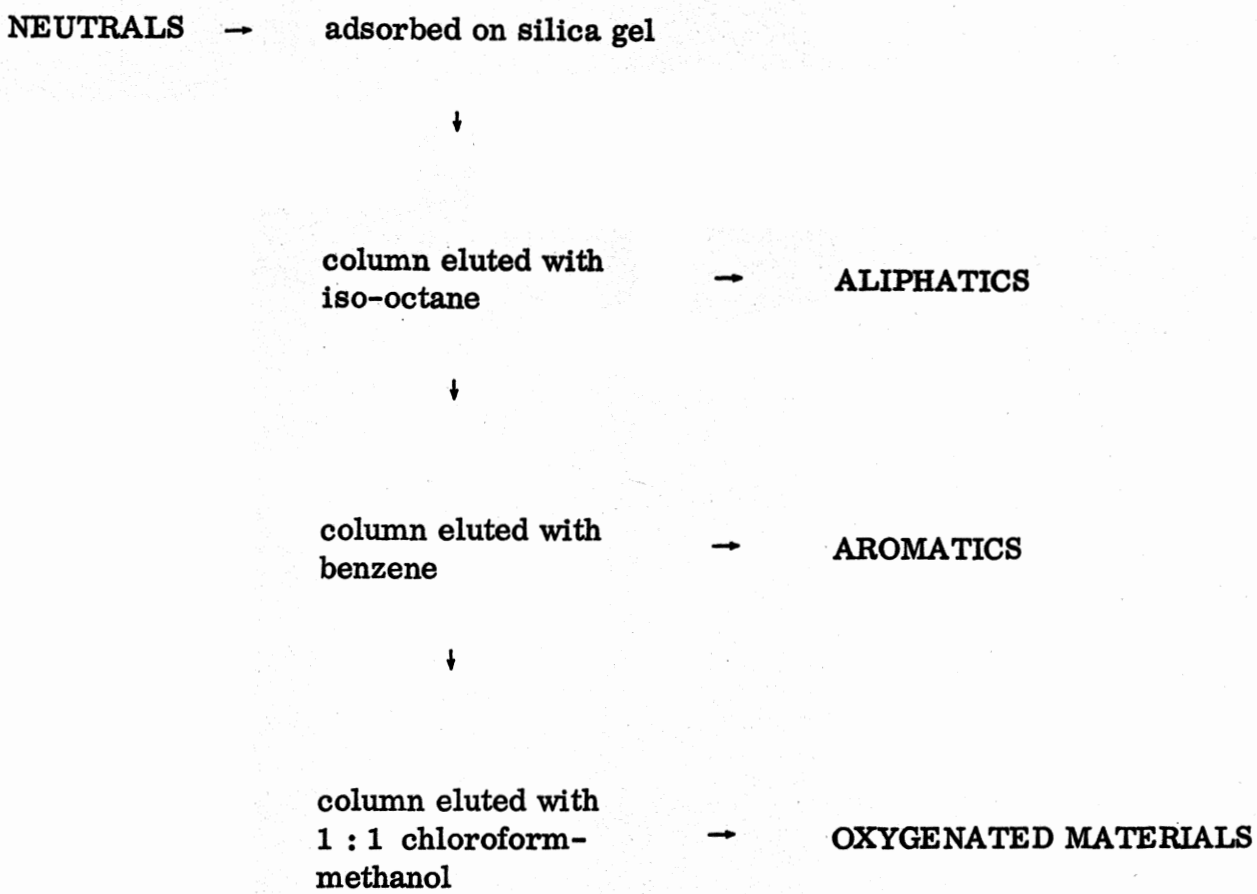


Figure 7

Chromatographic Separation of Neutrals

a high of 289 mg/l CAE, were subjected to biological action using a seed concentration of 1000 mg/l mixed liquor suspended solids (45 to 65 per cent volatile suspended solids) which had been suspended in a concentrated BOD dilution water. A seed blank was used with each Warburg run in order to measure endogenous activity, and a glucose sample was employed to check the activity of the seed microorganisms.

5. Toxicity Studies.

Preliminary toxicity studies were performed using rainbow trout, Salmonidae gairdnerii, (average length 18 cm, average weight 46 grams) obtained from the Meramec Springs Fish Hatchery and bluegreen perch, Percidae flavescens, (average length 7.5 cm, average weight 9.1 grams) obtained from the Indian Trails Fish Hatchery. The fish were transported to the laboratory in five gallon polyethylene bottles with ice and oxygen added to keep the water temperature low and to maintain an adequate dissolved oxygen level in the container. The toxicity studies were performed in a constant temperature, walk-in room maintained at $11 \pm 0.5^{o}C$ for the trout studies and at $16 \pm 0.5^{o}C$ for the perch studies. The fish were acclimated in the laboratory in 15 gallon glass aquaria for a period of ten days. These aquaria were equipped with an aeration apparatus consisting of a two foot length of 0.25 inch diameter polyethylene tubing with 6 pin holes through which air was pumped with a Precision air-vacuum pump (Model 35). The fish were fed Purina Trout Chow each day except for the test period and the 48 hours preceeding it. The water in the aquaria was changed daily, and the aquaria were cleaned with soap and water each day.

Since the approximate toxicity of these organic extracts to rainbow trout

and perch was not known, exploratory bioassay tests were conducted (48, p. 551) using solutions which contained only CCE or CAE and solutions containing both CCE and CAE in their natural occurring proportions to evaluate any synergistic effects. Ten and three liter volumes of water containing the organics were used for the trout and perch studies, respectively. A control jar was also used which contained only dilution water. Three acclimated fish were placed in each jar and observed periodically over a 24 hour period.

On the basis of the results of the exploratory tests, an appropriate range of concentrations was selected for the preliminary bioassay studies. The concentrations constituted a logarithmic series as recommended in Standard Methods (48, p. 553). Three samples, each with a volume of 18 liters, were prepared for the trout study while seven were prepared for the perch study. A larger number of samples would have been desirable for the trout study, but due to the limited number of fish available three samples were all that could be used. A control unit was also employed to detect any disease or physical unfitness of the test fish or faulty procedure. For the trout study, five test fish were transferred to each of the three test containers and the control, while ten perch were placed in each of the seven containers and control unit. The fish were observed continuously for the first two to four hours of the test, depending on their reaction. Thereafter, the observations were periodic but often enough to determine the number of fish killed at the end of 24, 48, and 96 hours.

6. Odor Studies.

Odor characterizations and threshold odor determinations were performed by a panel of six judges. The general procedure as outlined in Standard Methods

(48, p. 356) was followed in these determinations. A series of four training periods preceeded the actual odor determinations in which the panel was trained to detect different concentrations of odorous materials. Dilutions of the stock organic solutions were made with odor free water obtained by passing tap water through a Nuchar C-190 + 20 mesh activated carbon filter. The odor determinations were conducted at both 60 and 20°C to obtain the hot and cold threshold odor concentrations.

IV. PRESENTATION OF RESULTS

The results of this investigation are presented in two parts: (a) recovery of organic micropollutants and (b) characterization of organic micropollutants.

A. RECOVERY OF ORGANIC MICROPOLLUTANTS

Organic materials were concentrated and recovered from three water sources using four activated carbon filter runs. Two filter runs were made at Meramec Spring, one at the UMR well, and one at the City of Rolla well. The quantity and concentration of the organics recovered in each of the four filter runs are presented in Table V. The values shown in Table V have been corrected for the amount of organic material initially adsorbed on the activated carbon and the organics which may have been picked up from the polyethylene bags and sheets used to transport and dry the carbon (see Table IV, page 32). The correction for the blanks amounted, with one exception, to less than two per cent of the total organics recovered from the carbon; however, due to the very small quantity of organics recovered, the blank for the CCE* from the City of Rolla well was almost 20 per cent of the organics obtained.

The organic materials recovered from the two runs at Meramec Spring differed considerably in concentration, physical appearance, and characteristics, even though these runs were consecutive and were completed within a 35-day sampling period. The concentration of the organic materials in the spring water ranged from a low of 44 μg/l CCE in Run 1 to a high of 195 μg/l CAE** in Run 2.

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*Carbon chloroform extracts
**Carbon alcohol extracts

Table V

Quantity and Concentration of Organic Micropollutants Recovered from Subsurface Waters with Chloroform and Alcohol Elution

Sampling Location		Quantity of Organics Recovered grams			Concentration of Organics µg/l		
		CCE	CAE	Total	CCE	CAE	Total
Meramec Spring							
Run 1	Unit 1	14.9463	23.2431	38.1894	30.45	47.55	78.00
	Unit 2	5.2186	19.2606	24.4792	10.80	39.60	50.40
	Unit 3	1.2022	10.6580	11.8602	2.46	21.75	24.20
	Total	21.3671	53.1617	74.5288	43.71	108.90	152.60
Run 2	Unit 1	25.8823	38.3738	64.2561	51.70	76.40	128.10
	Unit 2	10.9617	36.7020	47.6637	21.85	73.30	95.15
	Unit 3	9.3303	22.8560	32.1863	18.61	45.75	64.34
	Total	46.1743	97.9318	144.1061	92.16	195.45	287.60
UMR Well							
	Unit 1	2.5788	4.3325	6.9113	4.80	8.04	12.84
	Unit 2	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -
	Unit 3	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -
	Total	2.5788	4.3325	6.9113	4.80	8.04	12.84
City of Rolla Well							
	Unit 1	0.0731	2.0609	2.1340	0.074	2.08	2.15
	Unit 2	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -
	Unit 3	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -	- 0 -
	Total	0.0731	2.0609	2.1340	0.074	2.08	2.15

Although the total volume of water filtered in each run at Meramec Spring was almost the same, the total chloroform soluble materials recovered from Run 2 (46 grams) were more than twice the CCE from Run 1 (22 grams), while the total alcohol soluble materials from Run 2 (98 grams) were somewhat less than twice the CAE from Run 1 (53 grams). Sproul and Ryckman (17) have reported values of 98 μg/l CCE and 230 μg/l CAE recovered from an unpolluted stream in Missouri. These values are about twice the concentration of the CCE and CAE from Run 1 at Meramec Spring, while the concentration of CCE from Run 2 was about equal to the CCE value reported by Sproul and Ryckman and the concentration of the CAE was almost 85 per cent of this value.

During the first eight days of Run 1 at Meramec Spring the water had a low turbidity (less than 100 Jackson Candle units) and color (10 units). Periods of heavy rainfall occurred during the final eight days of Run 1 and the first six days of Run 2 and resulted in a significant increase in the flow of the spring. The water was highly turbid (turbidity greater than 1000 units) during this period. During the final 12 days of Run 2 the water did not contain a large amount of turbidity (less than 100 units), but it was highly colored (more than 70 units). The sudden increase in flow and turbidity of the spring with the periods of heavy rainfall in the area, and the persistance of the colored water after the flow had returned to normal, would indicate that feeder routes of the spring were supplied, to a large extent, by surface waters from the immediate area. Since karst terrain prevails in the infiltration area of Meramec Spring, it is probable that the rain water passing through the soil picked up considerable quantities of organic materials from the soil and carried them to the spring water. The larger number

of sampling days in Run 2 with highly colored water would explain the larger recovery of organics in this run.

The concentration of the organic materials recovered from both wells (Table V) was very low as compared to that obtained from Meramec Spring. Almost 17 times as much total material was recovered from Meramec Spring Run 1 as from the UMR well and almost 37 times as much as from the City of Rolla well. Approximately 30 times as much CCE and 4 times as much CAE were recovered from the UMR well as were recovered from the City of Rolla well, even though almost twice the volume of water was filtered at the City of Rolla well than was filtered at the UMR well. The City of Rolla well was considerably deeper than the UMR well and originated from a different rock formation (see Table I, page 22). Also, the possibility that the UMR well was receiving surface contamination, as evidenced by increased amounts of turbidity and coliform organisms, may account for the higher recoveries. However, another possible explanation for the higher recoveries is the breakdown of the organics adsorbed on the carbon which could have occurred during the run at the City of Rolla well because of the long sampling period employed.

As can be seen from the data presented in Table V, large amounts of organic materials were recovered with Units 2 and 3 of both Runs 1 and 2 at Meramec Spring, while no detectable chloroform or alcohol soluble materials were recovered from Units 2 and 3 of either of the well runs. The relative recovery efficiencies of the three units employed in the Meramec Spring runs are shown in Table VI. The majority of the chloroform soluble materials

Table VI

Relative Recovery Efficiencies of the Three
Activated Carbon Filter Units Employed at Meramec Spring

Run	Organic Extracts	Unit 1	Unit 2		Unit 3		
		% of Total†	% of Unit 1	% of Total	% of Unit 1	% of Unit 2	% of Total
1	CCE	70	35	24	8	23	6
	CAE	44	83	36	46	55	20
	Combined Extracts	51	64	33	31	48	16
2	CCE	56	42	24	36	85	20
	CAE	38	96	38	60	62	24
	Combined Extracts	44	74	33	50	67	23

† Total amount recovered in filter run (sum of recoveries from Units 1, 2, and 3).

obtained in Run 1 were recovered with Unit 1, while only about one-half of those obtained in Run 2 were recovered with Unit 1 and approximately equal amounts of the remaining organics were recovered with Units 2 and 3. Almost equal quantities of CAE were recovered with Units 1 and 2 in Run 2. The relatively high recoveries of CAE with Unit 2 in both runs indicate that the adsorptive capacity of the carbon in Unit 1 had been exceeded and organics were being carried to Unit 2. The significant recoveries of CCE and CAE with Unit 3 in both runs further emphasize that these organics are complex compounds, as indicated by their selective adsorption on the carbon.

In an attempt to investigate the quantity of organic materials, other than those which were chloroform and alcohol soluble, in selected samples the activated carbon which had been extracted with chloroform and alcohol was eluted with acetone and benzene. The concentrations of the acetone and benzene soluble materials are shown in Table VII. The numbers "1" and "2" refer to the order at which acetone and benzene were used to elute the carbon. The two different series (Acetone-1, Benzene-2 and Benzene-1, Acetone-2) were used in order to establish an optimum system for complete recovery of the organic micropollutants. To facilitate comparison, the concentrations of the chloroform and alcohol extractable materials are also shown in Table VII. It may be seen that chloroform and alcohol elution did not remove all the organics from the carbon. Although additional extraction of the activated carbon from Meramec Spring Run 2 Unit 1 with an Acetone-1 Benzene-2 series yielded a quantity of organics equivalent to only four per cent of the total CCE and CAE, elution of the activated carbon from the City of Rolla well run yielded a quantity of organics equivalent to 11 per cent

Table VII

Concentration of Organic Micropollutants Recovered From
Subsurface Waters with Chloroform, Alcohol, Acetone, and Benzene Elution

Sampling Location	Elutant Employed					
	Chloroform	Alcohol	Acetone-1†	Benzene-2†	Benzene-1†	Acetone-2†
	Concentration of Organics μg/l					
Meramec Spring Run 2						
Unit 1	51.70	76.40	4.04	0.82	1.98	1.58
City of Rolla Well						
Unit 1	0.07	2.08	0.17	0.14	1.17	1.35
Unit 3	-0-	-0-	0.16	0.12	1.05	1.10

† Numbers "1" and "2" refer to the order in which Acetone and Benzene were used as elutants.

of the total CCE and CAE. When the acetone-benzene sequence was reversed, the benzene and acetone soluble organics dropped to two per cent of the total organics from Meramec Spring, while the concentrations of the benzene and acetone soluble materials from the City of Rolla well increased by a ten fold amount and were higher than the concentrations of the CCE and CAE. In addition, while no detectable CCE or CAE were recovered from Unit 3 of the City of Rolla well run, acetone and benzene elution yielded concentrations of organics which were approximately equal to those obtained from Unit 1. Myrick and Ryckman (6) have reported for Missouri River water values in excess of 100 and 80 μg/l for the Acetone-1 and Benzene-2 soluble materials, respectively, in addition to 80 μg/l CCE and 320 μg/l CAE previously recovered. The Acetone-1 and Benzene-2 values reported by Myrick and Ryckman were, respectively, approximately 125 per cent of and equal to the corresponding CCE concentration. Therefore, the recoveries with acetone and benzene, relative to the CCE, obtained by these investigators were considerably higher than those obtained in either the City of Rolla well or Meramec Spring study.

B. CHARACTERIZATION OF ORGANIC MICROPOLLUTANTS

The physical characteristics of the organic extracts recovered from the various sources are presented in Table VIII. The color, form, and odor of the CCE and CAE from the spring and two wells were considerably different.

It should be pointed out that the extracts from the City of Rolla well were not placed in solution or subjected to any further characterization because of the very small amounts of materials which were recovered. The CCE from UMR well exhibited a lower solubility than the corresponding CAE when the "VirTis"

Table VIII

Characterization of Organic Micropollutants
Physical Appearance and Solubility in Water

Extract	Physical Characteristic			Solubility in Water, mg/l	
	Color	Form	Odor	Directly Soluble*	With Concentration**
Meramec Spring					
Run 1 Unit 1					
CCE	dark brown	solid	chemical or musty	140	280
CAE	dark brown	highly viscous	flowery or sweet	300	340
Run 2 Unit 1					
CCE	dark brown	solid	chemical or musty	140	280
CAE	dark brown	highly viscous	flowery or sweet	300	340
UMR Well					
Unit 1					
CCE	light brown	slightly viscous	medicinal	100	170
CAE	dark brown	slightly viscous	musty	120	150
City of Rolla Well					
CCE	light brown	slightly viscous	medicinal	†	†
CAE	light yellow	highly viscous	chemical	†	†

*Extract placed in solution by mixing with a "VirTis" mixer for 24 hours at an estimated 8000 rpm.
**Solution of extract concentrated with Rinco evaporator.
†Due to the small amount of extract recovered, no solutions were prepared.

mixer was used, but the CCE were more soluble than the CAE when solutions of higher concentration were made using the Rinco evaporator. This characteristic was not observed with the extracts from the spring.

The organic extracts were further subjected to various analyses and determinations in an attempt to partially characterize these materials.

1. Elemental Chemical Analyses.

Elemental chemical analyses were performed on several selected samples by a commercial laboratory to provide data on the chemical composition of the extracts. The results of these analyses are given in Table IX. A second analysis was performed on some of the extracts in order to evaluate the accuracy and reproducibility of the values reported by the laboratory. Because the results of the two analyses varied somewhat (less than eight per cent variation except for the sulfur values for the UMR well CCE where a variation of 30 per cent was noted), the values for each analysis and the average of the values are shown in Table IX. Due to the cost involved, analyses were not performed on all the extracts; however, the samples selected for analysis were considered representative of the organics recovered in this investigation. In general, the alcohol soluble materials were more oxygenated, contained less carbon and more nitrogen than the chloroform soluble materials. The phosphorus content of all extracts, except one, was less than 0.4 per cent; the one exception was the CAE from Meramec Spring Run 2 Unit 2 which contained 2.8 per cent phosphorus. The sulfur content of the extracts was very small (less than 0.2 per cent) except for the CCE from UMR well Unit 1 which was close to 28 per cent.

Table IX

Characterization of Organic Micropollutants
Elemental Chemical Analysis†

Extract	Carbon		Hydrogen		Oxygen		Sulfur		Phosphorus	Nitrogen
	Analysis	Average	Analysis	Average	Analysis	Average	Analysis	Average	Analysis	Analysis
Meramec Spring										
Run 1 Unit 1										
CCE	57.9 60.0	59.0	6.83 7.10	6.96	24.2 24.2	24.2	< 0.1	--	0.36	0.68
CAE	50.8 51.6	51.2	6.44 6.61	6.52	36.8 35.2	36.0	< 0.1	--	0.35	2.53
Run 2 Unit 2										
CCE	62.5	--	7.40	--	24.0	--	0.20	--	0.22	0.54
CAE	46.3	--	7.47	--	36.6	--	0.13	--	2.80	2.04
UMR Well										
Unit 1										
CCE	50.8 54.2	52.5	6.43 7.06	6.74	14.7	--	32.0 23.7	27.8	0.22	0.32
CAE	42.9	--	6.81	--	36.0	--	< 0.1	--	0.24	1.36

On the basis of the chemical composition, the following empirical formulae were developed.

	CCE	CAE
Meramec Spring		
Run 1 Unit 1	$C_{4.5}H_{6.8}O_{1.5}N_{0.05}P_{0.01}$	$C_{4.2}H_{6.4}O_{2.36}N_{0.18}P_{0.01}$
Run 2 Unit 2	$C_{5.2}H_{7.4}O_{1.5}N_{0.04}P_{0.01}$	$C_{3.9}H_{7.5}O_{2.29}N_{0.14}P_{0.09}S_{0.01}$
UMR Well	$C_{4.2}H_{6.4}O_{0.92}N_{0.02}P_{0.01}S_{1.0}$	$C_{3.6}H_{6.8}O_{2.25}N_{0.10}P_{0.01}$

Goncharova and Datska (25) have reported an empirical formula of $C_{4.4}H_{7.0}O_{2.3}N_{0.23}$ for an organic extract recovered from subsurface waters in several European countries by liquid-liquid extraction of the water with benzene. This formula compares favorably with the formula developed for the CAE from Meramec Spring Run 1 Unit 1.

2. Chemical Oxygen Demand Determinations.

Chemical oxygen demand values were determined using the dilute dichromate reflux method and are presented in Table X. The theoretical amount of oxygen required for the complete breakdown of several organic extracts to carbon dioxide and water was calculated using the elemental chemical composition data given in Table IX and are also shown in Table X. The theoretical oxygen demand (TOD) ranged from a high of 2.01 mg oxygen per mg extract for the CCE from Meramec Spring Run 2 Unit 2 to a low of 1.48 mg oxygen per mg extract for the CAE from the same filter. With one exception, the COD did not vary from the

Table X

Characterization of Organic Micropollutants
Oxygen Demand

Extract	Theoretical† mg O_2/mg Extract	Chemical†† mg O_2/mg Extract	Chemical†† % Theoretical	5-day Biochemical†† mg O_2/mg Extract	5-day Biochemical†† % Chemical
Meramec Spring					
Run 1 Unit 1					
CCE	1.85	1.37	74	0.260	19.0
CAE	1.52	1.49	98	0.229	15.3
Unit 2					
CCE		1.48		0.129	8.7
CAE		1.30		0.104	8.0
Unit 3					
CCE		1.35		0.150	11.1
CAE		1.40		0.140	10.0
Run 2 Unit 1					
CCE		1.32		0.176	12.9
CAE		1.31		0.215	16.5
Unit 2					
CCE	2.01	2.00	99	0.164	8.2
CAE	1.48	1.40	95	0.191	13.6
Unit 3					
CCE		1.72		Not Determined	
CAE		1.94			
UMR Well					
Unit 1					
CCE	1.72	1.64	95	-0-	-0-
CAE	1.55	1.46	94	-0-	-0-

†Calculated from emperical formulae based on values shown in Table VIII.
††Results have been duplicated.

TOD by more than ten per cent. The exception was the COD of the CCE from Meramec Spring Run Unit 1 which was 74 per cent of the TOD. Sproul and Ryckman (17) have reported COD values of 1.56 and 0.98 mg oxygen per mg extract for the CCE and CAE, respectively, recovered from an unpolluted Missouri stream water. The COD value for the CCE compares favorably with the COD value obtained for the corresponding organics recovered from all runs in this investigation, but the COD value for the CAE is much lower than the corresponding values. Myrick and Ryckman (6) have reported higher COD values for CCE from Missouri River water (2.1 to 2.3 mg oxygen per mg extract), while the COD values for the CAE from the same water (1.2 to 1.4 mg oxygen per mg extract) were close to the values obtained for the subsurface water extracts.

3. Biodegradability Studies.

In order to evaluate more completely the oxidation characteristics of the organic extracts, biodegradability studies were performed using a Warburg respirometer. The data obtained in these studies are shown in Table A-1, Appendix A, and Table XI, and are plotted in Figure 8. For each organic extract evaluated, three different concentrations were studied. Two Warburg runs, each with duplicate vessels,were made for each test concentration. In each Warburg run duplicate vessels, each containing 500 mg/l glucose, were employed in order to evaluate the activity of the seed organisms. A total of 12 runs were made. The values reported in Table A-1 for each extract test concentration are, therefore, averages of the uptake values measured in four vessels, while the glucose values are averages of the uptakes determined from 24 vessels. The seed microorganisms, obtained from an activated sludge unit, were first concentrated and then suspended in a phosphate-

Table XI

Characterization of Organic Micropollutants
Biodegradability Studies

Time		M-1 U-1		M-1 U-2		M-1 U-3		M-2 U-1		M-2 U-2		UMR Well Unit 1	
Hrs.	Glucose	CCE	CAE	CCE	CAE	CCE	CAE	CCE	CAE	CCE	CAE	CCE	CAE
		Oxygen Uptake, mgO_2 per mg Substance											
1	0.014	0.006	0.007	0.004	0.006	0.009	0.008	0.006	0.014	0.014	0.008	0	0
2	0.032	0.015	0.015	0.015	0.008	0.014	0.010	0.010	0.032	0.032	0.020	0	0
3	0.046	0.026	0.025	0.022	0.010	0.019	0.013	0.017	0.046	0.040	0.026	0	0
4	0.060	0.030	0.035	0.030	0.013	0.025	0.018	0.020	0.056	0.050	0.034	0	0
5	0.074	0.037	0.045	0.035	0.018	0.030	0.021	0.025	0.065	0.050	0.034	0	0
6	0.090	0.042	0.056	0.042	0.020	0.036	0.026	0.030	0.077	0.065	0.045	0	0
10	0.134	0.064	0.080	0.058	0.030	0.048	0.039	0.048	0.100	0.088	0.072	0	0
24	0.240	0.120	0.148	0.086	0.052	0.066	0.076	0.090	0.130	0.128	0.132	0	0
30	0.270	0.160	0.183	0.091	0.066	0.072	0.085	0.104	0.140	0.130	0.141	0	0
48	0.320	0.185	0.211	0.100	0.080	0.090	0.108	0.135	0.182	0.142	0.164	0	0
52	0.322	0.191	0.218	0.104	0.080	0.092	0.110	0.140	0.189	0.144	0.169	0	0
72	0.330	0.228	0.227	0.110	0.091	0.110	0.125	0.160	0.206	0.153	0.180	0	0
85	0.350	0.241	0.227	0.118	0.095	0.122	0.129	0.167	0.211	0.157	0.183	0	0
96	0.400	0.250	0.228	0.120	0.098	0.140	0.130	0.170	0.215	0.160	0.188	0	0
110	0.432	0.257	0.229	0.124	0.099	0.148	0.137	0.171	0.215	0.160	0.190	0	0
120	0.440	0.260	0.229	0.129	0.104	0.150	0.140	0.176	0.215	0.164	0.191	0	0

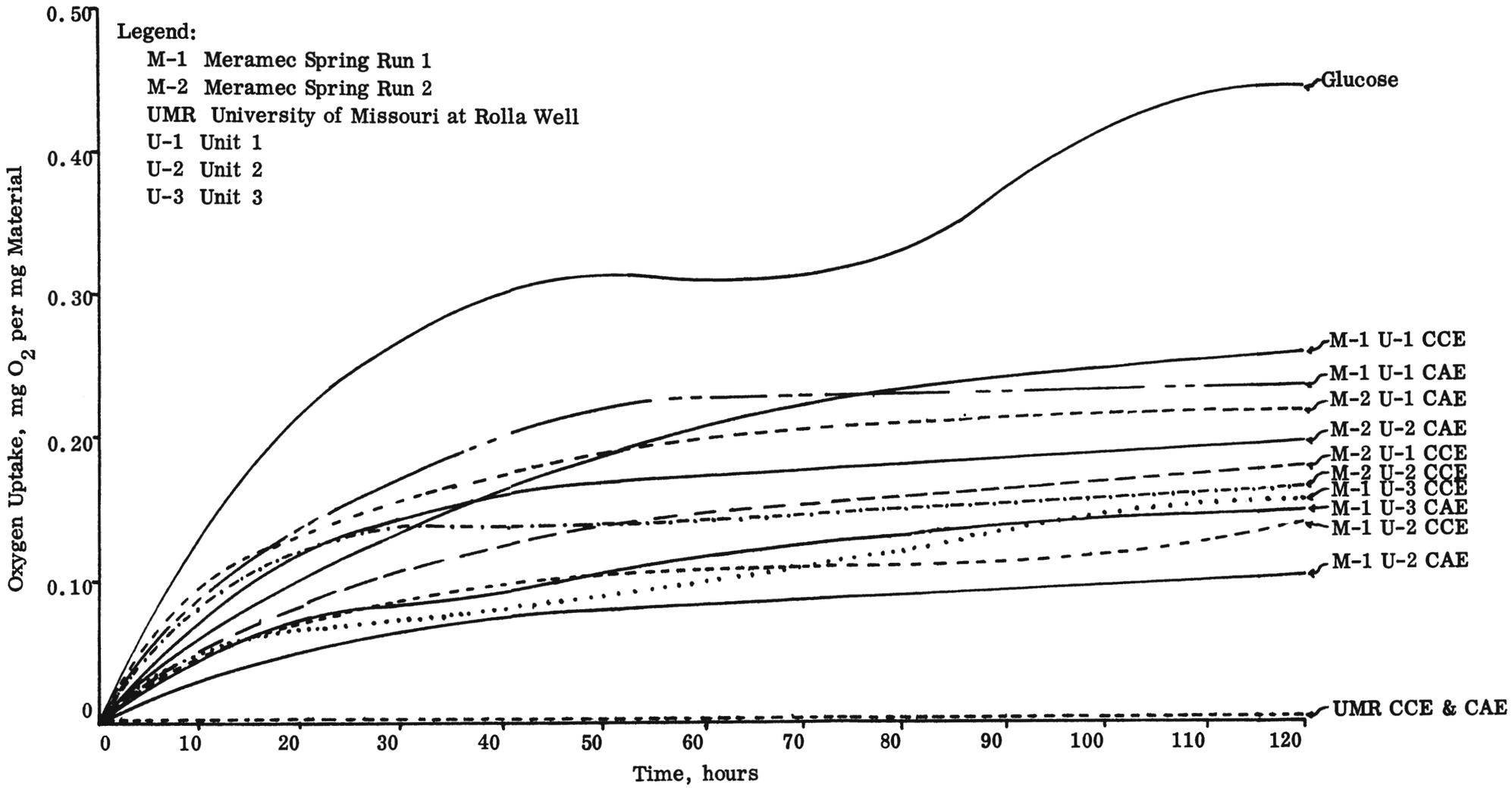

Figure 8

Biodegradability Studies on the Organic
Micropollutants from Missouri Subsurface Waters

buffered dilution water containing inorganic trace elements as nutrients. Several investigations were performed to evaluate the necessity of using the buffer and nutrients and indicated that the activity of the cells was substantially increased in their presence. The concentration of the seed microorganisms in the reaction vessel was 1000 mg/l.

The values presented in Table XI and plotted in Figure 8 represent net oxygen uptakes due to the presence of the organic materials. These values were obtained from the biodegradability data (Table A-1) by substracting the amount of the blank from the total oxygen uptake and then dividing this net uptake by the sample concentration. The 5-day BOD values for the extracts were read from the curves in Figure 8 and are presented in Table X.

The biodegradability of the extracts varied considerably from a low of zero mg 5-day BOD per mg extract for the UMR well extracts (CCE and CAE) to a high of 0.260 mg 5-day BOD per mg extract for the CCE from Meramec Spring Run 1 Unit 1. This value is equivalent to 19 per cent of the COD of this extract as compared to the value of 44 per cent of the COD observed for glucose. Sproul and Ryckman (17) have reported that the 10-day BOD for a CCE from an unpolluted stream was two per cent of its COD, while the 10-day BOD of a combined CCE and CAE extract from the same source was six per cent of the COD. These figures are considerably lower than the 5-day BOD values in Table X.

4. Solubility Partitioning.

Solubility partitioning was used to separate chloroform extracts from Meramec Spring and the UMR well into various groups based on their solubility in ether under different conditions of pH. The results of solubility separations performed on several extracts are shown in Table XII. These values are an

Table XII

Characterization of Organic Micropollutants
Solubility Partitioning

Extract	Fraction, %							
	Ether Insolubles	Water Solubles	Weak Acids	Strong Acids	Bases	Amphoterics	Neutrals	Non-Recoverable
Meramec Spring								
Run 1 Unit 1								
CCE	10.0	31.0	8.5	11.8	0.77	0.91	11.8	25.2
CAE	>99.0	--	--	--	--	--	--	--
Run 2 Unit 1								
CCE	2.1	27.0	13.6	13.4	0.83	0.35	14.0	28.7
CAE	>99.0	--	--	--	--	--	--	--
UMR Well Unit 1								
CCE	6.4	15.0	6.8	11.5	1.50	1.60	21.4	35.8
CAE	>99.0	--	--	--	--	--	--	--

average of four separations. An attempt was also made to separate several alcohol soluble extracts; however, these were insoluble in ether and no further separation was possible. The predominating group in the spring samples was the water soluble fraction, while the neutrals were the largest group in the well sample. The neutrals were further broken down by column chromatography; the results of this separation are presented in Table XIII. The aromatic and oxygenated fractions were about equal in the extracts from Meramec Spring Run 1 Unit 1, while the oxygenated fraction was the largest group in the extracts from Meramec Spring Run 2 Unit 1. The aliphatic group predominated in the UMR well extract. The extracts recovered by Myrick and Ryckman from river water, which are shown in tabular form on page 13, exhibited very different solubility partitioning characteristics. The neutral and water soluble fractions showed the greatest variations. The quantity of the neutrals in the river extracts was almost three times the quantity of the neutrals in the spring samples (both runs) but only one and one-half the amount of the neutrals in the well sample. The water solubles from both runs at the spring were over twice the amount of the water solubles found in the river water, while the water solubles from the UMR well were 20 per cent less than those from the river water. Considerable variation is seen also in the groups composing the neutral fraction. The neutrals from the river water extracts were 88 per cent oxygenated materials and 7 per cent aromatic materials, while the neutrals from the Meramec Spring Run 1 Unit 1 extracts were 37 per cent oxygenated and 37 per cent aromatic materials and the neutrals from the UMR well were 20 per cent oxygenated and 20 per cent aromatic materials.

Table XIII

Characterization of Organic Micropollutants
Column Chromatographic Separation of Neutrals

Extract	Fraction, %			
	Aliphatics	Aromatics	Oxygenated Materials	Non-Recoverables
Meramec Spring				
Run 1 Unit 1 CCE	16.1	36.9	37.0	10.0
Run 2 Unit 1 CCE	22.0	24.5	50.5	23.0
UMR Well Unit 1 CCE	48.5	19.6	21.6	10.3

5. Toxicity Studies.

The acute toxic effects of the extracts from Meramec Spring Run 2 Unit 1 were evaluated with bioassay studies using rainbow trout and bluegreen perch as test fish. Since the relative toxicity of either the CCE or the CAE was not known, exploratory bioassays were performed. These tests showed that the CCE were not toxic to either test fish at concentrations up to 270 mg/l and the CAE were not toxic to either fish at concentrations up to 320 mg/l. Concentrations higher than these were not possible due to the limited solubility of the extracts. Exploratory bioassays were then performed using the CCE and CAE combined in their naturally occurring proportions (CCE/CAE : 1.0/1.48) to evaluate any synergistic effects of the extracts. Based on the results of the exploratory tests, preliminary bioassay studies were performed with the trout and perch using the combined CCE and CAE and the results are presented in Table XIV. While the individual extracts exhibited no toxicity at concentrations greater than 250 mg/l, the 24-hour median tolerance limit (TLm) of the combined extracts was 130 mg/l for the trout and 137 mg/l for the perch. The 48- and 96-hour TLm values for the trout were the same as the 24-hour value (130 mg/l) and the corresponding TLm values for the perch were 120 and 114 mg/l, respectively. These values were obtained by interpolation from the curves in Figures 9 and 10. It may be pointed out that Sproul and Ryckman (17) reported that the combined extracts obtained from an unpolluted stream at a concentration of 100 mg/l showed no effect on test fish (2 inch trout) in six days.

If the TLm values are expressed in terms of fish size, mg/l per gram fish weight, the 24-, 48-, and 96-hour TLm values for trout are 0.56 and the 24-, 48-, and 96-hour TLm values for the perch are 1.51, 1.32, and 1.25,

Table XIV

Characterization of Organic Micropollutants
Toxicity Studies

Test Fish	Number of Fish Used	Ave. Size	Extract	Concentration mg/l	% Surviving 24 hr.	48 hr.	72 hr.	96 hr.	120 hr.	TLm, mg/l 24 hr.	48 hr.	96 hr.
				Control	100	100	100	100	100			
Rainbow Trout	5	18 cm, 46 g	Meramec Spring Run 2 Unit 1	100	100	100	100	100	100	130	130	130
				160	20	20	20	20	20			
				250	0	0	0	0	0			
				Control	100	100	100	100	100			
				56	100	90	90	90	90			
				100	100	100	90	90	90			
Bluegreen Perch	10	7.5 cm, 9.1 g	Meramec Spring Run 2 Unit 1	135	40	20	10	0	0	137	120	114
				155	40	20	10	0	0			
				180	30	20	10	0	0			
				210	0	0	0	0	0			
				240	0	0	0	0	0			

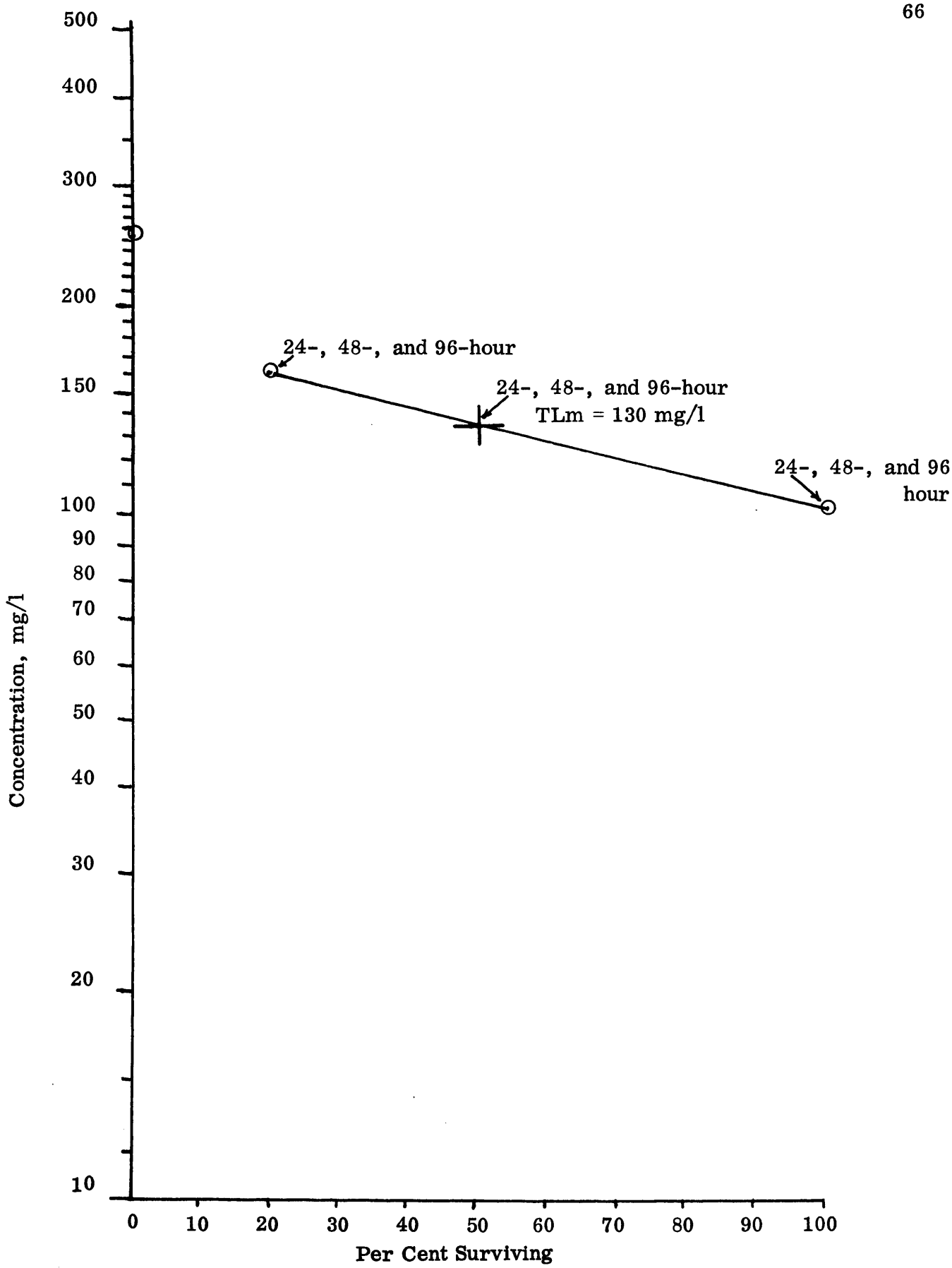

Figure 9

Characterization of Organic Micropollutants
Toxicity Studies - Rainbow Trout

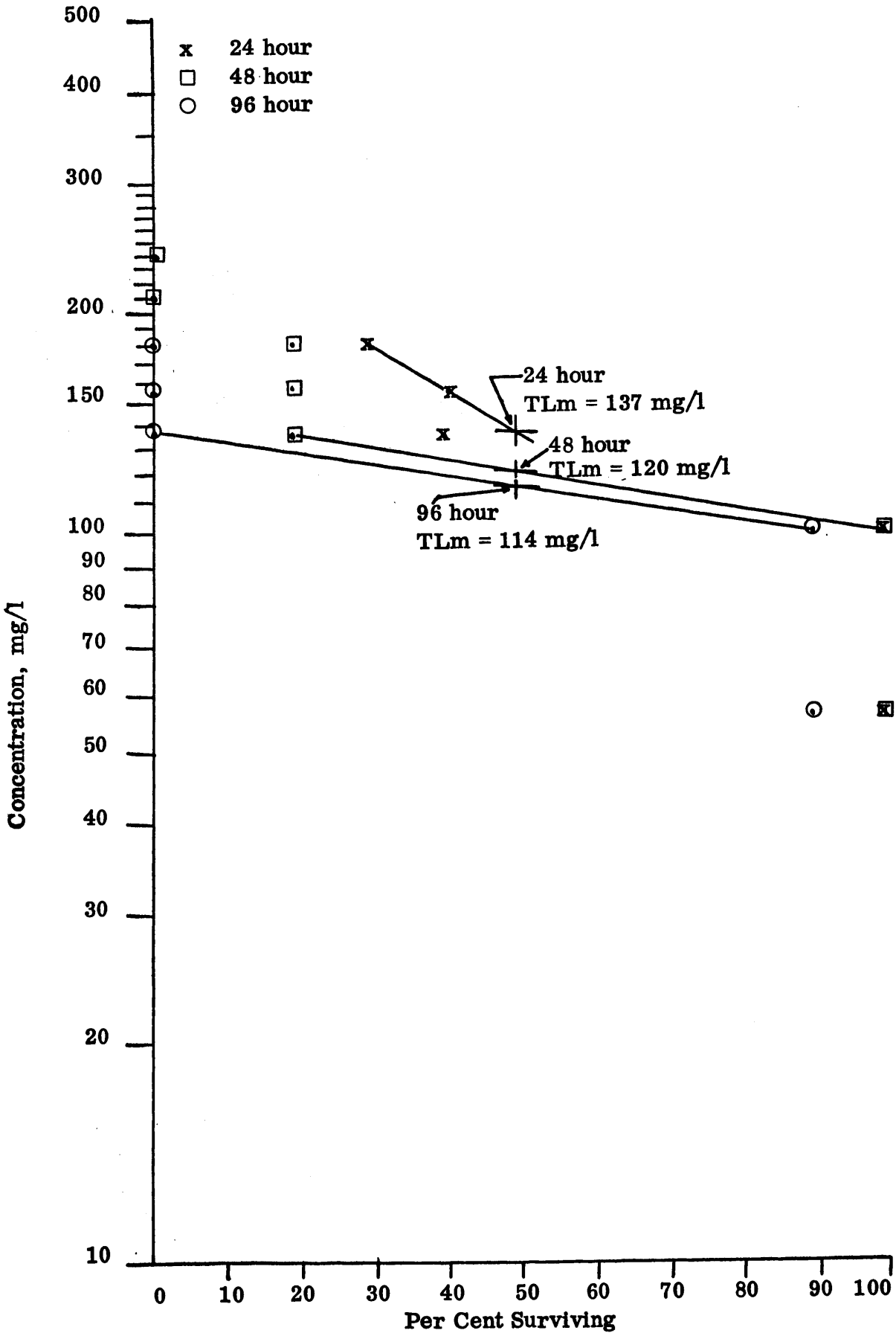

Figure 10
Characterization of Organic Micropollutants
Toxicity Studies - Bluegreen Perch

respectively. In the studies reported by Sproul and Ryckman, the extract concentration of 100 mg/l was equivalent to about 5. 5 mg/l per gram of trout weight. The test fish showed little or no reaction to the higher concentrations of the organics for the first four hours, but after six hours the test fish in the highest concentration began violently swimming from the bottom to the top of the test container. In the final two hours before death, the test fish exhibited little motion except for unsteady, violent gill movements.

6. Organoleptic Determinations.

The odor potentials of several of the extracts were determined and are shown in Table XV. Also presented are the characteristic odors of each extract as determined by the panel members. The extracts from Units 2 and 3 exhibited different odor potentials and characteristics than the extracts from Unit 1. Sproul and Ryckman (17) reported threshold odor concentration values of 220 μg/l CCE and 390 μg/l combined CCE and CAE recovered from an unpolluted stream. The CCE from Meramec Spring Run 1 Unit 2 were the most odorous of the extracts studied and exhibited a musty or flowery characteristic odor. The next most odorous extracts were the CAE from Meramec Spring Run 1 Unit 2 which exhibited a chemical characteristic odor.

Two odors were studied, the 20^{o}C or cold odor and the 60^{o}C or hot odor. The CCE from Meramec Spring Run 1 Unit 2 and Run 2 Unit 1 and Unit 2 exhibited a 2 to 1 ratio of cold odor to hot odor concentrations. The CCE and CAE of Meramec Spring Run 1 Unit 1 and the UMR well Unit 1 exhibited a considerably greater ratio of cold to hot odor concentration.

Table XV

Characterization of Organic Micropollutants
Organoleptic Determinations

Extract	Threshold Odor μg/l 20°C C†††	Threshold Odor μg/l 60°C H†††	Odor Characteristics, % of Judges† Musty C	Musty H	Earthy C	Earthy H	Medicinal C	Medicinal H	Chemical C	Chemical H	Flowery C	Flowery H	Miscellaneous†† C	Miscellaneous†† H
Meramec Spring														
Run 1 Unit 1														
CCE	2310	203	67	67									33	33
CAE	3400	151	50	33							33	33	17	33
Unit 2														
CCE	110	52	50	33								33	50	33
CAE	166	35			33				33	33		33	33	33
Unit 3														
CCE	564	110	50	33	33					50			17	17
Run 2 Unit 1														
CCE	226	127	33	33			33					33	33	33
CAE	553	140	50	33			33					33	17	33
Unit 2														
CCE	110	58	33	33							50	33	33	33
CAE	267	140	33	33		33					33		33	33
UMR Well														
Unit 1														
CCE	15500	880						33	33	33				
CAE	28300	3440	33	33			33	33	33					

†Six judges reporting specific characteristic.
††Any characteristic not reported by more than 17 per cent of judges.
†††C - cold odor
H - hot odor

V. DISCUSSION OF RESULTS

As can be seen from the results presented in the preceeding section, organic micropollutants were found in all the spring and well waters sampled. The organics were recovered by passing a large volume of water serially through three large capacity activated carbon filters and eluting the carbon with different solvents. Two filter runs were made at Meramec Spring and one each at the University of Missouri at Rolla and the City of Rolla wells. The concentrations of the organic materials recovered from the two well waters were very small compared to the concentration found at Meramec Spring. Also, while Unit 1 of each well run recovered all the organics obtained from the well waters, the materials recovered with Units 2 and 3 of both runs at Meramec Spring accounted for a large portion of the total quantity of organics found in the spring waters.

A predetermined volume of water, which was selected after considering the works of other investigators (see Table II, page 30), was passed through the filters. Of these investigators, the Taft Center group used only one activated carbon filter. On the other hand, the Washington University group, working with Missouri River water, employed two filters in series with the pH of the water being reduced before it was charged on the second filter, and have reported larger recoveries with the second unit than with the first. They attributed this larger amount to the lowered pH of the water charged on the second filter. When it is considered, however, that a smaller concentration of organics was found in both runs at Meramec Spring than what was reported for the Missouri River and that the total amount of material recovered with Units 2 and 3 of each Meramec

Spring run was equal to or greater than that recovered with the corresponding Unit 1, it seems likely that a part of the organics recovered from the second filter of the Missouri River studies would have been recovered even without pH adjustment of the water. The findings of the present study further indicate that in the previous investigations where only one or two filters were employed, a large quantity of organics could have been leached from the filter or not even adsorbed on the carbon and, therefore, not recovered. Although the subsurface and surface water extracts possess different characteristics, this difference in character is not believed to be totally responsible for the sizeable additional recoveries obtained with Units 2 and 3 of this study.

The concentrations of the organic materials recovered with each unit of Runs 1 and 2 at Meramec Spring are presented in Figure 11. Units 2 and 3 of Runs 1 and 2 recovered about 27 and 33 μg/l, respectively, less than their preceeding unit. Thus, while in Run 1 it is estimated that the three units effectively removed all the organics from the water, it appears that a significant quantity of materials were not recovered with the three units in Run 2 and a fourth unit would have been necessary for essentially total recovery.

In order to avoid exceeding the adsorptive capacity of the filters, their effluents could have been monitored to detect an increase in the concentration of organics. Monitoring could have been undertaken by liquid-liquid extraction of grab samples of the effluents from the units; however, the time involved and the volume of water required to give a measureable amount of material did not render this method applicable. Also, if the organics were present at high enough concentrations to impart an odor to the water, odor determinations could have been

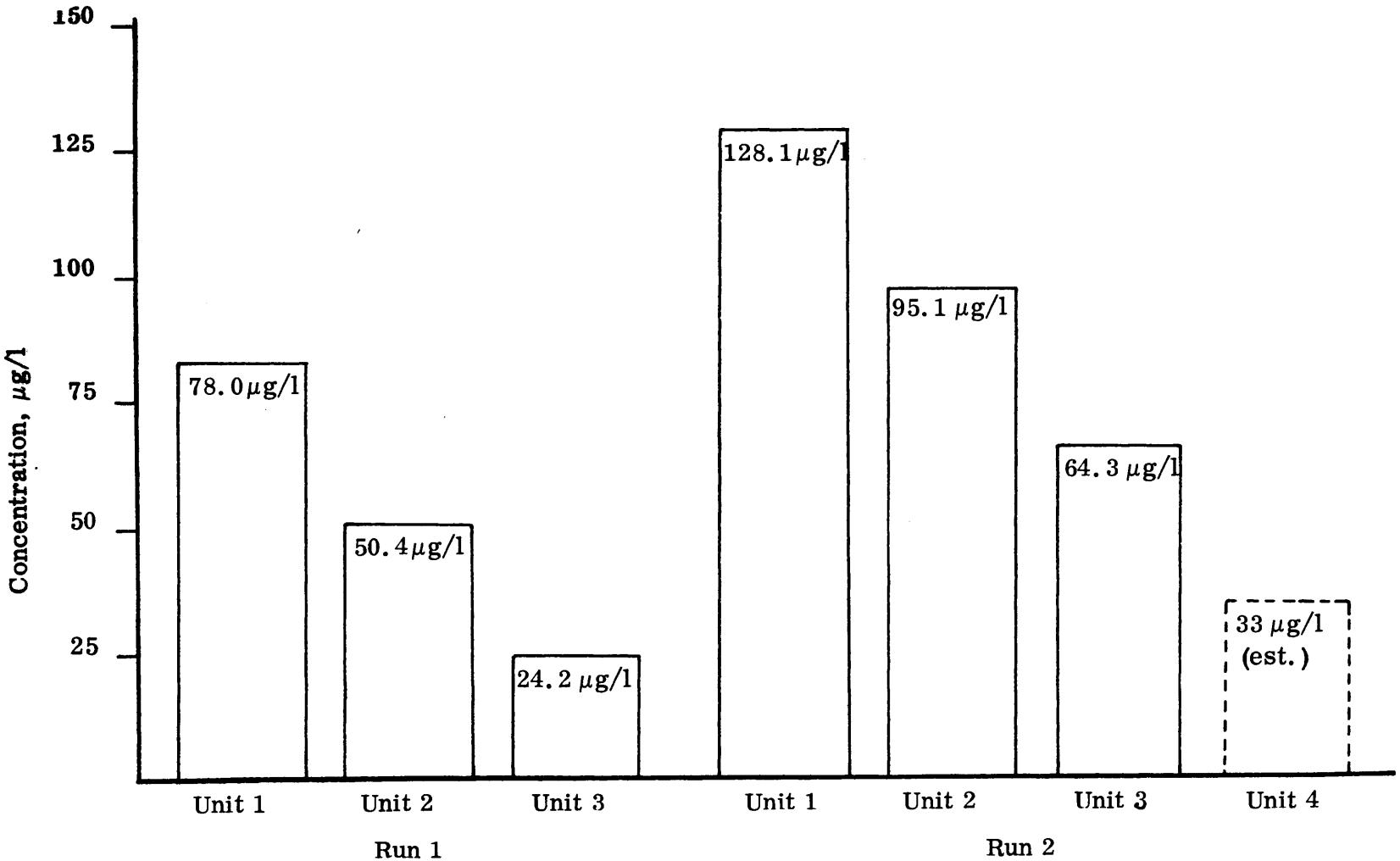

Figure 11

The Concentration of Organic Materials (CCE & CAE) Recovered With Each of the Three Filter Units at Meramec Spring

used to monitor the effluents. Another technique which might be used is quantitative analysis with a gas liquid chromatograph; this instrument has the sensitivity required to detect small concentrations of organics and requires a short time for the analysis.

A summary of the major characteristics of the organic micropollutants which were determined in this investigation is presented in Table XVI. The various materials appear to be different compounds as evidenced by their odor potential, chemical oxygen demand, and biodegradability. The UMR well extracts exhibited considerably different characteristics from the spring extracts; the organics from the well water were not biodegradable, exhibited a lower odor potential, and, in general, were more chemically oxidizable.

One of the basic assumptions in this investigation was that the chloroform soluble materials possessed the greatest odor potential. Although this assumption was validated, it was found that the CAE, while possessing a lower odor potential, were present in larger concentrations and, consequently, were also significant as odor causing materials. The odor potential of the benzene and acetone soluble extracts was not evaluated because of the small amounts of these materials which were recovered.

In the Warburg respirometer studies using a high concentration of activated sludge seed organisms (1000 mg/l biological solids), it was found that the organics from the UMR well (both CCE and CAE) caused no increase in oxygen uptake over the endogenous respiration of the seed. This would indicate that these extracts were not biodegradable even at the high concentrations tested. On the other hand, the presence of the spring extracts (both CCE and CAE) did result in a net oxygen

Table XVI

Summary of the Characteristics of the
Organic Micropollutants Recovered from Subsurface Waters

Extract	Characteristic: Concentration μg/l	Threshold Odor Concentration μg/l	Chemical Oxygen Demand mg O_2/mg Extract	5-Day Biochemical Oxygen Demand % COD
Meramec Spring				
Run 1 Unit 1				
CCE	30.45	2,310	1.37	19.0
CAE	47.55	2,400	1.49	15.3
Unit 2				
CCE	10.80	110	1.48	8.7
CAE	39.60	166	1.30	8.0
Unit 3				
CCE	2.46	564	1.35	11.1
CAE	21.75			
Run 2 Unit 1				
CCE	51.70	226	1.32	12.9
CAE	76.40	553	1.31	16.5
Unit 2				
CCE	21.85	110	2.00	8.2
CAE	73.30	267	1.40	13.6
Unit 3				
CCE	18.61		1.72	
CAE	45.74		1.94	
UMR Well Unit 1				
CCE	4.80	15,500	1.64	0.0
CAE	8.04	28,300	1.46	0.0
Rolla Well Unit 1				
CCE	0.07			
CAE	2.08			

uptake above that due to the seed. Different concentrations of the extracts were tested, and it was found that the oxygen consumed per mg of extract did not vary with the concentration. This would indicate that at the concentrations tested (which were several thousand times the naturally occurring concentrations) these materials were biodegradable to a limited extent. The 5-day oxygen uptake of the spring extracts, which ranged from 8 to 19 per cent of the corresponding COD values, is significantly higher than the 2-hour oxygen uptake value of 4.2 to 4.5 per cent of COD reported by Myrick and Ryckman (6) for Missouri River water extracts. These investigators performed 6-hour Warburg respirometer studies with 2000 mg/l acclimated biological solids in the reaction vessel. Although the length of these Warburg runs was considerably shorter than that used in the present study, Myrick and Ryckman noted that the additional oxygen uptake for the last four hours of the runs was very small. They also reported that after ten days only three per cent of the COD had been biochemically oxidized by a low concentration of microorganisms (standard BOD test conditions).

In an attempt to elucidate more fully the biodegradability of the extracts, an attempt was made to calculate the rate constant, k, of the biochemical oxidation reaction (49, p. 272) for several extracts from the corresponding oxygen uptake values presented in Table XI, page 58. Because the ultimate oxygen uptake was not determined, the constant k was computed using several sets of uptake values at different time intervals. The procedure used and assumptions made in calculating k are described in Appendix B and the resulting values are listed in Table B-1. The values of k determined for each extract varied and, in some cases, showed a significant deviation from the average. Although some deviation was to be

expected due to experimental error, it is believed that the magnitudes observed cannot be justified on the basis of experimental error alone and it would appear that the kinetics of the biological oxidation of the organic materials did not follow the standard BOD equation. In addition, the constants for several of the extracts were significantly higher than that determined for glucose and all values were greater than the 0.1 to 0.2 range accepted for the breakdown of sewage (49, p. 280). Further investigations need to be undertaken to determine the kinetics of the biochemical breakdown of the organics including any possible toxic effects the organics might exert on the microorganisms. One such effect, which could cause an increased oxygen utilization when the organics are present, is their acting as uncouplers of the oxidative phosphorylation scheme in the mitochondria of the microbial cells. Mahler and Cordes (50) have reported that various substituted phenols, especially 2,4-dinitrophenol and pentachlorophenol, as well as phenylhydrazones and arsenate have the ability to stimulate respiration without net cell growth. Since the spring extracts are very complex and contain some aromatic substances (see Table XIII, page 63), the possibility exists that compounds which have the capability of acting as uncouplers could be present in the extracts and, when high concentrations of the extracts are used, their concentration could be high enough to effect metabolic reactions.

The acute toxicity of two of the extracts obtained at Meramec Spring (Run 2 Unit 1) was evaluated using fish in bioassay studies. The extracts exhibited considerable synergistic toxicity in their naturally occurring ratio of CCE/CAE : 1/1.48. The fish were not disected after death and, consequently,

the affected organ was not ascertained. The toxicity of other organics was not evaluated due to the large quantity of materials required for a bioassay study and the short period of time which the correct size fish were available.

In summary, based on the characteristics determined in this investigation, the extracts from Missouri subsurface waters appear to be considerably different from the compounds recovered from both a Missouri stream receiving no industrial or domestic pollution (17) and the Missouri River (6) which receives a large amount of domestic and industrial pollution; and could be of considerable importance because of their odor potential and possible toxic effects if present in higher concentrations, even though they may be partially broken down biologically at these concentrations.

VI. CONCLUSIONS

Based on the findings of this study, the following conclusions can be drawn:

1. Organic micropollutants were found in Missouri subsurface waters (both spring and well) and were present at great depths below the surface of the earth. In some waters, their concentrations were comparable to the concentration of organic materials recovered in a similar manner from an unpolluted Missouri surface stream.
2. The organic materials recovered from subsurface waters exhibited considerably different characteristics from those from surface waters. In addition, the materials extracted from each of the three filters in series possessed different characteristics.
3. The spring extracts showed sizeable oxygen uptake in Warburg respirometer studies indicating limited biodegradability, while the deep well extracts did not show any uptake indicating that they were not biodegradable. In all cases, the extracts did not inhibit the activity of the unacclimated seed organisms.
4. The chemical oxygen demand of all but one extract was within 90 per cent of the theoretical oxygen demand as calculated from empirical formulae developed from elemental chemical analyses.
5. Preliminary acute toxicity studies with both chloroform and alcohol spring extracts (CCE and CAE) showed that these organics exhibited considerable synergistic toxic effects to both rainbow trout and bluegreen perch, but

were not individually toxic even at concentrations which were twice as great, or more, than the sum of the concentrations of the combined extracts.

6. Organoleptic studies performed on the spring and well extracts established that these materials did exhibit an odor potential and that the spring extracts were considerably more odorous than the well extracts. The threshold odor concentrations of the CCE were, in all cases, lower than the corresponding CAE concentrations.

7. The number of filters in series required for effective recovery of the organics from subsurface water depended on the source of the water and the concentration of the organics in the water. One filter was adequate for the well waters, while three or four were needed for the spring water.

8. The subsurface organic micropollutants, at the presently occurring concentrations, do not pose an immediate problem to water quality either from an aesthetic or a health point of view. However, these materials do exhibit odor causing and acute toxic characteristics at higher concentrations and, therefore, further research needs to be undertaken to evaluate their long-term toxicity and develop a means of removing these materials from the water.

VII. RECOMMENDATIONS FOR FUTURE RESEARCH

Organic micropollutants were found in some subsurface waters at significant concentrations. It was further established that these materials possessed odor and acute toxicity potentials when present in high concentrations. During the course of the characterization of these organic materials, the following areas meriting further investigation were uncovered.

1. The characteristics of organic materials other than those chloroform and alcohol soluble need to be determined. Significant concentrations of acetone and benzene soluble organics were recovered, especially from well waters.
2. The long-term toxic effects of the organic extracts and the affected organs should be established. Because of the limited amount of these materials, a long-term fish bioassay procedure using recirculation of the test solution or some other "microbioassay" technique needs to be developed. Also, the use of enzyme-assays with the Warburg respirometer should be evaluated as a toxicity measuring technique.
3. The removal of the organic micropollutants from the water by physical or chemical means needs to be investigated, as well as the biodegradability of these materials with an acclimated microbial system and the kinetics of the biochemical oxidation of the extracts.
4. The compounds composing the organic materials need to be determined. This would require sophisticated separation and identification techniques, such as thin layer and gas liquid chromatography. Identification of specific

compounds would make possible the correlation of the toxic and organoleptic characteristics with definite components of the extracts.

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APPENDICES

APPENDIX A

Biodegradability Studies

The biodegradability studies were performed using a Warburg respirometer. The oxygen utilized by seed microorganisms in attempting to break down the organic extracts was measured. The reaction vessel-manometer systems were calibrated using the water method as described by Ludwig, et al. (50). The oxygen uptake, in mg/l,was calculated by the following equation which relates the manometer reading in millimeters to the oxygen uptake in mg/l:

Oxygen uptake = Flask Constant X Manometer Reading

Duplicate runs were made for each extract at three different concentrations. In each run, two reaction vessels for each extract concentration, a glucose sample, a barometer, and a seed blank were used. The glucose sample and seed blank were included in order to evaluate the activity and measure the endogenous respiration of the seed microorganisms. The barometer was used to compensate for the change in the manometer reading due to atmospheric pressure changes. Because of the number of extracts involved, 12 Warburg runs were performed measuring the oxygen utilization over a 120-hour period. The oxygen uptake data are presented in Table A-1. These values are averages for the four vessels used for each extract. Also presented are average uptake values for the glucose and seed blank samples. These values represent an average for 24 vessels; the greatest deviations from the average for the glucose and seed blank were 12 and 8 per cent, respectively. A large number of readings

Table A-1

Oxygen Uptake Data for Biodegradability Studies

Oxygen Uptake, mg/l†

Time	Meramec Spring 1 Unit 1						Meramec Spring 1 Unit 2						Meramec Spring 1 Unit 3						Seed Blank, mg/l
	CCE, mg/l			CAE, mg/l			CCE, mg/l			CAE, mg/l			CCE, mg/l			CAE, mg/l			
Hrs.	100	150	200	100	200	250	100	200	230	100	200	289	50	100	180	75	150	200	1000
1	2.6	2.8	3.2	2.8	3.3	4.0	2.3	3.0	3.0	2.7	2.4	4.0	2.5	3.0	3.6	2.6	3.2	3.7	2.0
2	5.0	5.6	6.4	5.0	6.4	7.8	5.0	6.8	6.9	4.2	5.1	5.7	4.1	4.8	6.0	4.0	5.0	5.4	3.4
3	6.6	7.9	9.0	6.5	9.0	11.4	6.2	9.4	9.2	5.0	6.0	7.0	5.0	5.8	7.4	5.0	5.9	6.6	3.9
4	7.8	9.4	10.7	8.3	11.9	15.0	7.9	10.9	11.7	6.1	7.5	8.9	6.0	7.4	9.8	6.0	7.5	8.3	4.8
5	11.2	13.1	15.0	11.9	16.6	21.0	11.0	14.6	15.5	9.2	11.0	12.6	9.0	10.6	12.8	8.9	10.5	11.4	7.4
6	12.0	14.3	16.1	13.4	19.0	24.3	12.0	16.1	17.4	10.0	11.8	13.7	9.5	11.4	14.3	9.7	11.7	13.0	7.8
10	19.4	23.0	24.7	22.0	29.0	36.0	19.0	25.0	26.0	16.0	19.0	22.0	15.1	17.5	22.0	15.9	18.6	20.8	12.9
24	38.4	44.1	50.0	41.0	56.1	70.2	34.6	43.0	46.0	31.2	35.8	41.2	29.0	33.0	38.0	32.0	37.0	41.1	26.1
30	47.0	55.0	64.0	49.4	67.6	87.0	41.0	49.2	51.9	38.0	42.0	52.0	35.0	38.0	44.0	37.0	44.0	48.0	31.0
48	66.6	75.7	86.1	69.0	90.0	112.0	58.0	68.0	71.0	56.0	64.0	71.0	52.0	57.0	65.0	55.0	64.0	70.0	48.2
52	71.0	80.1	90.3	74.1	95.4	118.0	62.0	73.0	77.0	61.0	68.0	75.0	56.0	61.0	69.0	60.0	69.0	74.0	51.6
72	90.0	101.0	113.0	90.0	123.0	151.0	78.0	89.0	93.0	76.1	85.0	93.0	73.0	78.0	88.0	77.0	86.0	93.0	67.5
85	103.0	114.0	127.0	100.0	133.0	161.0	89.0	101.0	104.0	87.2	96.0	104.0	83.0	90.0	100.0	86.0	94.0	103.0	77.8
96	112.0	124.0	136.0	109.0	142.0	169.0	98.0	112.0	114.0	96.0	106.0	114.0	93.0	100.0	115.0	95.0	105.0	112.0	86.0
110	123.0	137.0	149.0	120.0	153.0	181.0	109.0	122.0	125.0	107.0	117.0	126.0	104.0	112.0	124.0	107.0	117.0	125.0	97.0
120	135.0	148.0	163.0	132.0	164.0	194.0	122.0	135.0	139.0	120.0	130.0	139.0	117.0	124.0	138.0	120.0	130.0	137.0	109.0

†Average of four reaction vessels, except seed which is average of 24 vessels.

Table A-1 (Cont.)

Oxygen Uptake Data for Biodegradability Studies

Oxygen Uptake, mg/l†

Time	Meramec Spring 2 Unit 1						Meramec Spring 2 Unit 2						UMR Well Unit 1						Glucose, mg/l
	CCE, mg/l			CAE, mg/l			CCE, mg/l			CAE, mg/l			CCE, mg/l			CAE, mg/l			
Hrs.	75	100	171	100	170	260	150	175	250	95	166	200	9	50	105	25	75	100	500
1	2.5	2.6	3.0	3.2	5.0	5.0	3.4	3.5	4.1	2.8	3.3	3.5	1.9	1.9	1.8	1.9	2.0	1.9	9.1
2	4.2	4.4	5.1	6.2	7.8	12.0	6.3	7.0	8.2	5.4	6.7	7.4	3.4	3.4	3.6	3.5	3.4	3.5	20.2
3	5.3	5.7	6.1	8.1	10.2	15.7	7.6	8.1	10.0	6.5	8.3	9.1	3.9	3.8	4.0	3.9	4.0	3.9	26.5
4	6.4	6.9	8.5	10.4	12.6	19.0	9.3	10.1	12.4	8.0	10.5	11.7	4.7	4.7	4.6	4.6	4.6	4.7	35.0
5	9.2	9.9	11.7	14.0	16.0	24.0	12.7	13.6	16.3	10.3	14.2	15.6	7.1	7.3	7.3	7.3	7.2	7.3	44.5
6	10.0	11.0	12.9	15.6	18.5	28.0	13.6	14.6	17.6	12.0	15.2	16.8	7.5	7.5	7.7	7.6	7.7	7.7	53.0
10	16.6	18.0	22.0	23.0	38.8	38.0	21.0	21.1	26.0	19.7	24.9	27.4	13.1	13.0	12.8	13.1	12.9	13.0	80.0
24	33.0	36.0	42.0	39.0	45.0	59.0	37.6	39.5	46.1	38.6	47.7	52.3	26.0	26.0	25.8	26.0	26.1	26.2	146.3
30	39.0	41.0	49.0	45.0	50.0	67.0	43.0	45.0	50.0	44.4	54.3	59.2	31.0	31.5	32.0	31.3	32.0	31.1	164.0
48	58.0	61.0	71.0	63.0	73.0	95.0	61.0	63.0	70.0	63.7	75.5	80.8	49.0	48.2	48.0	48.7	48.3	48.1	210.0
52	62.0	66.0	75.0	70.0	78.0	101.0	64.3	67.0	73.0	67.8	79.7	85.4	51.5	51.6	51.1	51.6	51.4	51.7	212.5
72	79.0	83.0	94.0	88.0	96.0	120.0	82.0	84.0	90.0	84.3	98.0	104.0	68.0	67.6	67.4	67.8	67.4	67.6	232.0
85	89.0	94.0	106.0	99.0	108.0	132.0	92.0	94.0	100.0	95.1	108.2	114.0	77.3	77.5	77.2	77.4	77.1	77.3	253.0
96	98.0	103.0	115.0	107.0	116.0	142.0	100.0	103.0	110.0	104.0	117.0	124.0	85.7	85.8	86.0	85.7	85.6	85.9	284.0
110	109.0	114.0	128.0	118.0	130.0	152.0	111.0	114.0	121.0	115.0	128.6	135.0	95.9	97.0	96.4	96.5	96.9	97.0	316.0
120	122.0	125.0	140.0	130.0	140.0	165.0	123.0	127.0	135.0	127.0	140.0	147.0	109.0	110.0	108.0	109.0	110.0	110.0	330.0

†Average of four vessels, except glucose which is average of 24 vessels.

were taken over the 5-day test period of each run and, consequently, it was not possible to take all the readings at the same time intervals. Therefore, values obtained at selected intervals which were the same for all runs are presented in Table A-1.

In order to evaluate both the biodegradability and any toxic effects of the extracts, a range of concentrations was used, including the highest obtainable value. Because of the difference in solubility, the extracts were not evaluated at the same concentrations and, consequently, the oxygen uptake values presented in Table A-1 cannot be compared on the mg/l basis. The oxygen utilization was, therefore, converted from mg/l to mg oxygen per mg extract. This was done by assuming that the net oxygen uptake due to the presence of the organics was equal to the difference between the total oxygen uptake for the sample and the seed blank. The net oxygen uptake as mg/l was then divided by the extract concentration yielding a value in terms of mg oxygen consumed per mg extract used. The oxygen uptake values presented in Table A-1 were reduced by the oxygen uptake values of the blank employed in the corresponding run, not the average value of the blank given in Table A-1, and then converted to mg oxygen per mg extract by dividing the net oxygen uptake by the extract concentration. These reduced values are presented in Table XI, page 58, and plotted in Figure 8, page 59. A sample calculation is presented as follows:

Sample	Meramec Spring Run 1 Unit 1 CCE	
Concentration, mg/l	Test 1 - 200	Test 2 - 200
Time, hours	120	120
Oxygen Uptake of sample, mg/l	165	157
Oxygen Uptake of blank, mg/l	110	108
Net Oxygen Uptake		
mg/l	55	49
mg oxygen per mg extract	0.275	0.245
Average	0.260	

APPENDIX B

OXYGEN UPTAKE RATE CONSTANT CALCULATION

The oxygen uptake rate constant, k, was calculated for several extracts and glucose from the biodegradability data shown in Table XI, page 58 using the following equation (49, p. 272):

$$y = L(1 - 10^{-kt}) \tag{1}$$

where, y is the oxygen uptake at any time t

L is the ultimate oxygen uptake

k is the oxygen uptake rate constant

Since the ultimate oxygen uptake was not determined, k was calculated on the basis of two uptake values y_1 and y_2, at times t_1 and t_2. The equation relating these terms was derived as follows:

$$y_1 = L(1 - 10^{-kt_1}) \qquad y_2 = L(1 - 10^{-kt_2})$$

therefore,

$$L = \frac{y_1}{1 - 10^{-kt_1}} = \frac{y_2}{1 - 10^{-kt_2}}$$

or,

$$y_1 (1 - 10^{-kt_2}) = y_2 (1 - 10^{-kt_1})$$

$$y_1 - y_1 (10^{-kt_2}) = y_2 - y_2 (10^{-kt_1})$$

$$y_2 - y_1 = y_2 (10^{-kt_1}) - y_1 (10^{-kt_2})$$

$$\frac{y_2 - y_1}{y_1} = \frac{y_2}{y_1} (10^{-kt_1}) - 10^{-kt_2} \tag{2}$$

The constant k was evaluated by making trial and error substitutions in equation 2. It was calculated using several different sets of times in order to determine if the oxygen uptake data followed equation 1. The times which were chosen for these calculations were such that at the lower time any lag period had passed and at the upper time the majority of the 5-day oxygen uptake had occurred. Also, the two times in any one set were chosen such that both points did not fall on the flat part of the oxygen uptake curve. The k values obtained are presented in Table B-1. Also shown in this table are the average value of k for each sample and the maximum deviation from the average. A sample calculation is as follows:

Sample: Glucose

t_1 = 1/4 day $\qquad$ y_1 = 0.09 mg O_2/mg glucose

t_2 = 3 days $\qquad$ y_2 = 0.33 mg O_2/mg glucose

$$\frac{y_2 - y_1}{y_1} = \frac{y_2}{y_1} (10^{-kt_1}) - 10^{-kt_2} \qquad (2)$$

Assume k = 0.55

$$\frac{0.33 - 0.09}{0.09} = \frac{0.33}{0.09} (10^{-0.138}) - 10^{-1.65}$$

$$2.66 = 3.67\ (0.728) - 0.02$$

$$2.66 = 2.67 - 0.02$$

$$2.66 = 2.65$$

Therefore k = 0.55

Table B-1

Oxygen Uptake Rate Constant Data for Organic Micropollutants Recovered from Meramec Spring and Glucose

Sample		k Computed Using Oxygen Uptake Values at: 1/6 and 3 days	1/6 and 2 days	1/4 and 3 days	1 and 2 days	1 and 3 days	2 and 3 days	Average	Max. Deviation %
Meramec Spring									
Run 1	Unit 1								
	CCE	0.37	0.46	0.28	0.45	0.32	0.36	0.37	24
	CAE	0.44	0.47	0.49	0.53	0.46	0.58	0.50	16
	Unit 2								
	CCE	0.83	0.93	0.84	0.86	0.66	0.52	0.79	34
	CAE	0.37	0.46	0.43	0.46	0.37	0.46	0.42	12
	Unit 3								
	CCE	0.68	0.85	0.69	0.58	0.40	0.37	0.60	42
	CAE	0.41	0.48	0.40	0.53	0.41	0.51	0.46	15
Run 2	Unit 1								
	CCE	0.33	0.42	0.36	0.48	0.51	0.40	0.41	24
	CAE	0.83	0.96	0.82	0.54	0.43	0.47	0.68	46
	Unit 2								
	CCE	1.03	1.13	0.96	0.66	0.79	0.57	0.86	34
	CAE	0.55	0.60	0.50	0.71	0.58	0.53	0.58	22
Glucose		0.52	0.54	0.55	0.60	0.56	--	0.55	9

VITA

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He was married to Miss Karen Fusselman on December 19, 1964.